Disorders of the Serum Lipoproteins

I. Lipoprotein Deficiency States

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Serum lipids can be divided into four major classes, all of which are insoluble in water and exist in the serum as lipoprotein complexes. Table I gives the concentrations of the individual lipids in normal serum, the distribution of the major fatty acids esterified with each lipid, and the main lipoprotein carrier.

The non-esterified fatty acids (NEFA) which are bound to albumin are the most metabolically active of the lipids and have a rapid turnover, with a half-life of about 2 minutes. Their fatty acid composition reflects that of adipose tissue triglycerides from which they largely arise. Cholesterol, of which about two-thirds is esterified, is a major constituent of β -lipoprotein and contains a high proportion of linoleic acid. The phospholipids may be sub-divided into two main groups depending upon whether the molecule contains glycerol or sphingosine (Fig. 1); the glycerophosphatides have two fatty acids and a nitrogenous base and the main member of this group in serum is phosphatidyl choline (or lecithin). The sphingosine-containing lipids have only one fatty acid in each molecule; the major type is sphingomyelin. In normal serum about 65% of the total phospholipid is phosphatidyl choline and 20% is sphingomyelin. Triglycerides are formed by the esterification of glycerol with three fatty acids (Fig. 1). Their serum lipoprotein carrier varies according to the source of the triglyceride. Exogenous (dietary) triglycerides are transported as chylomicrons whose fatty acid pattern therefore reflects that of the dietary fat. Endogenous triglycerides which are synthesized in the liver, either from NEFA coming from adipose tissue or from carbohydrate, are transported by pre- β -lipoprotein and their fatty acid pattern reflects partly that of adipose tissue triglycerides and partly that of lipogenesis.

The serum lipoproteins may also be divided into four major classes and the simplest classification derives from their electrophoretic characteristics. Fig. 2 shows the patterns obtained after paper

electrophoresis and subsequent staining of the strip for lipid (Salt and Wolff, 1957); in the normal fasting subject two bands are seen: β -lipoprotein which has the mobility of β -globulin, and α -lipoprotein with the mobility of α_1 -globulin. When chylomicrons are present, as in the normal post-absorptive state, they remain at the point of application of the serum to the paper. The fourth band appears when there is excessive endogenous synthesis of triglyceride and, because it moves in advance of β -lipoprotein, it is known as pre- β -lipoprotein; it is not usually found in healthy children.

The composition of the individual lipoproteins can be determined after separation in a preparative ultracentrifuge, using a salt density gradient technique. Fig. 3 illustrates a tube in which such a density gradient has been established after ultracentrifugation: at the bottom of the tube are the lipoproteins of highest density (greater than 1.063) containing the greatest ratio of protein to lipid; these correspond to α -lipoproteins in paper electrophoretic terms. Also in this fraction are all the other serum proteins including serum albumin and the albumin bound NEFA. The middle fraction with density 1.063–1.019 corresponds to β -lipoprotein; the top fraction with density less than 1.019 contains very low density lipoproteins with the lowest ratio of protein to lipid and corresponds to pre- β -lipoproteins. Chylomicrons, which have a density less than that of plasma (0.96), may be separated by simple high speed centrifugation and are normally removed before ultracentrifugation. Analysis of the individual fractions shows that all lipoproteins contain a combination of cholesterol, phospholipids, and triglycerides: in chylomicrons triglycerides predominate; in pre- β -lipoprotein triglycerides are the major lipid but cholesterol is an important constituent; in β - and α -lipoproteins both cholesterol and phospholipids are present in considerable amounts, with cholesterol predominantly in the β -fraction and phospholipid in the α -fraction.

TABLE I
Normal Values for Fasting Concentrations and Fatty Acid Composition of Major Serum Lipids

Lipids	Serum Concentration (fasting)	% Distribution* of Major Fatty Acids (mean values)													Principal Lipoprotein Carrier
		C	16:0	16:1	18:0	18:1	18:2	20:0	22:0	20:4	23:0	24:0	24:1	22:5	
Non-esterified fatty acids (NEFA) ..	0.45-1.10 mEq/l.	31.9	4.8	12.5	33.2	12.9									Albumin
Triglycerides ..	40-125 mg./100 ml.	30.3	5.4	4.3	44.7	12.3									Chylomicrons (during fat absorption) Pre- β -lipoproteins (fasting)
Cholesterol, total ..	120-230 mg./100 ml.														β -lipoprotein
Cholesterol ester ..	80-170 mg./100 ml.	12.2	5.1		20.8	53.8			6.4						
Phospholipids-total	160-310 mg./100 ml.														α -lipoprotein
Individual phospholipids	% total														
phosphatidyl choline ..	64.5 $\pm$ 2.6	34.4			14.3	12.4	23.3			8.0				2.7	
sphingomyelin ..	20.0 $\pm$ 1.6	33.2			10.3	1.8	1.2	3.9	16.1	2.8	4.5	8.4	14.8		
phosphatidyl ethanolamine ..	5.5 $\pm$ 0.4	8.0			15.4	7.9	15.8	1.3		26.3				3.9	12.5
lysolecithin ..	9.5 $\pm$ 1.8	39.5			15.2	13.5	21.1			3.4				1.4	

* The difference from 100% is accounted for by minor components; nomenclature indicates number of carbon atoms and double bonds. Fatty acid data for NEFA, triglycerides, cholesterol ester, phosphatidyl choline, and sphingomyelin from our laboratory (Dr. I. Tamir and Mrs. S. Gould); for phosphatidyl ethanolamine and lysolecithin from Phillips and Dodge (1967). Percentage distribution of individual phospholipids (means $\pm$ SD) from our laboratory (Dr. E. Djardjorous).

FATTY ACIDS

eg. Palmitic acid (16 carbon atoms, no double bonds; C 16:0)

TRIGLYCERIDES : Glycerol + 3 Fatty acids (FA)

PHOSPHOLIPIDS : Two main classes

Glycerophosphatides : Glycerol + 2 Fatty acids

Sphingolipids : Sphingosine + 1 Fatty acid

CHOLESTEROL

CHOLESTEROL ESTER : Cholesterol + 1 Fatty acid

FIG. 1.—Major serum lipids.

FIG. 2.—Paper electrophoretic separation of serum lipoproteins.

The protein moieties of the individual lipoproteins (apoproteins) can be studied by immunochemical techniques and by analysis of their amino acid residues. The apoprotein of β -lipoprotein has been designated as B protein and its amino acid composition (Margolis and Langdon,

1966) and immunochemical characteristics (Lees, 1967) have been well defined. α -lipoprotein contains A protein which differs in both amino acid composition (Scanu, 1965) and immunochemical characteristics (Levy and Fredrickson, 1965) from B protein. Pre- β -lipoprotein contains both A and

Density (g./ml.)	S_f		Electrophoretic nomenclature	Immunochemical characteristics	Chemical composition (% distribution)			
					Protein	Triglyceride	Cholesterol	Phospholipids
0.96	>400		* Chylomicrons	?	2	82	9	7
	10-400	VLDL	Pre- β -lipoprotein	A + B	7	52	23	18
1.019	3-9	LDL	β -lipoprotein	B	21	9	47	23
1.063		HDL	α -lipoprotein	A	46	8	19	27
			Albumin + NEFA (+ remaining serum proteins)					

FIG. 3.—Ultracentrifugation of serum lipoproteins. Schematic representation of density gradient tube; identification and composition of individual lipoproteins. * Chylomicrons removed before ultracentrifugation by centrifugation at $10,000 \times g$ for 30 minutes. VLDL = very low-density lipoproteins; LDL = low-density lipoproteins; HDL = high-density lipoproteins. The S_f value is the flotation rate of the lipoprotein in sodium chloride medium of density 1.063 g./ml. at $26^\circ C$. expressed in Svedberg units (10^{-13} cm./sec./[dyne/g.]).

TABLE II
Classification of Lipoprotein Disorders

Lipoprotein Species Involved	Lipoprotein Deficiency States		Hyperlipoproteinaemias	
	Primary	Secondary	Primary	Secondary
Chylomicron		Malabsorption syndromes A- β -lipoproteinaemia	Hyperchylomicronaemia (familial fat-induced hypertriglyceridaemia; familial hyperlipaemia)	Primary and secondary elevation of pre- β -lipoprotein
β	Physiological in fetus and newborn A- β -lipoproteinaemia	Malabsorption syndromes Hyperthyroidism Chronic anaemia Liver cell failure	Hyper- β -lipoproteinaemia (familial hypercholesterolaemia)	Hypothyroidism Obstructive jaundice
Pre- β			Pre- β -lipoproteinaemia (CHO-induced hypertriglyceridaemia; endogenous hypertriglyceridaemia; some 'mixed' hyperlipaemias)	Conditions in which CHO is not freely available: diabetes mellitus glycogen storage disease starvation glucose/galactose malabsorption Nephrotic syndrome Infantile hypercalcaemia Progeria
α	Familial α -lipoprotein deficiency (Tangier disease)	Primary and secondary elevation of pre- β -lipoprotein Biliary obstruction Non-specific		Liver disease ? Steroid therapy Pregnancy

B proteins (Levy, Lees, and Fredrickson, 1966). The nature of the apoprotein of chylomicrons is still not certain (Fredrickson, Levy, and Lees, 1967); it is possible that several different proteins may be able to serve as stabilizers for chylomicrons.

Other substances transported either wholly or in part with the serum lipoproteins are carotenoids and fat-soluble vitamins. Vitamin A and β -carotene are transported from the intestinal cell in association with lymph chylomicrons, and in the serum the carotenoids are bound to β -lipoprotein. Vitamin E is similarly absorbed with chylomicrons and carried in the serum with β -lipoprotein.

Classification of Lipoprotein Disorders

Earlier classifications of lipoprotein disorders were largely based on terminology relating either to total concentrations of lipid in the serum, or to concentrations of the individual lipids, and thus it was difficult to define and distinguish different conditions which might yet have similar lipid abnormalities. Classification according to the major lipoprotein species involved has now been generally accepted as a more rational approach to the understanding of these disorders (Fredrickson *et al.*, 1967). This classification, however, also

has its limitations, as considerable heterogeneity undoubtedly exists among some of the hyperlipoproteinaemic states, notably those characterized by the presence of pre- β -lipoprotein. Nevertheless, until we have more precise knowledge of the basic defects responsible for the various disorders, this terminology is useful in directing our attention to the whole lipoprotein macromolecule. Table II gives a classification based on lipoprotein species, but also includes terminology based on specific lipids, such as familial hypercholesterolaemia, and exogenous- and endogenous-hypertriglyceridaemia. Part I of this review will deal with the lipoprotein deficiency states, both primary and secondary, and Part II will consider the hyperlipoproteinaemias.

Lipoprotein Deficiency States

Physiological lipoprotein deficiency of fetus and newborn. Cord blood serum lipids in the fetus at term are much lower than those of children or adults. β -lipoprotein is reduced to about 30% of the adult level, and α -lipoprotein to about 50% (Auerswald, Doleschel, and Müller-Hartburg, 1963). The mean total cholesterol concentration is about 70 mg./100 ml., phospholipid 110 mg./100 ml.,

triglycerides 30 mg./100 ml., and NEFA 0.38 mEq/l. (Mortimer, 1964; Crowley, Ways, and Jones, 1965; Persson and Gentz, 1966). Within the phospholipids there is a relative increase in sphingomyelin and decrease in phosphatidyl choline compared with the pattern in older children. Levels of linoleic acid are low. In early embryonic serum (P. Ways and J. K. Lloyd, unpublished) and in the premature fetus (Rafstedt, 1955) levels of cholesterol and phospholipid are similar to those found in the term infant.

After birth the serum NEFA concentration rises rapidly indicating lipolysis of stored fat, and remains high during the first month of life (Persson and Gentz, 1966). Cholesterol and phospholipid levels increase during the first week, and during this time the concentration of β -lipoprotein doubles or trebles (Lindquist and Malmcrona, 1960; Sweeney *et al.*, 1962).

A- β -lipoproteinaemia. This rare condition has now been described in about 35 cases and is characterized by absence of β -lipoprotein from the serum, steatorrhoea, acanthocytosis of the red cells, and the development in later childhood and adolescence of a pigmentary retinopathy and an ataxic neuropathy.

Genetics. The disease is inherited as an autosomal recessive and recent work suggests that the primary gene effect is not an inability to synthesize the B protein, but a defect in the formation of the complete β -lipoprotein macromolecule (Lees, 1967).

The heterozygotes have no clinical abnormalities, and in only one set of parents have low β -lipoprotein levels been detected (Salt *et al.*, 1960).

Clinical features and laboratory findings. In early infancy symptoms due to malabsorption predominate. Steatorrhoea is present from birth, and we have recently seen a patient in whom the diagnosis was made at the age of 4 weeks (M. D. Baber, J. K. Lloyd, A. S. Fosbrooke, M. M. Segall, and O. H. Wolff, unpublished). Secondary vitamin and mineral deficiencies such as hypoprothrombinæmia, rickets, and anaemia may occur. The first signs of a progressive retinitis and an ataxic neuropathy may appear at about 5 years. The retinopathy affects the macula as well as the periphery of the retina (Fig. 4), and impaired dark adaptation may be an early finding (Wolff, Lloyd, and Tonks, 1964) together with an abnormal electroretinogram (Rey, 1961). In a few patients mental retardation has been present since early childhood. In adult life malabsorption is less marked, but the patient becomes progressively crippled, and cardiac manifestations have been reported, one patient dying in cardiac failure (Sobrevilla, Goodman, and Kane, 1964).

The serum is clear; total cholesterol, phospholipid, and triglyceride concentrations are very low and no β -lipoprotein can be detected by paper electrophoresis, ultracentrifugation, or immunochemical techniques. Chylomicra do not appear after a meal containing fat. α -lipoprotein is reduced to about half its normal concentration, and

FIG. 4.—Retinopathy in α - β -lipoproteinaemia. Appearance of macula (left) and periphery (right) in a boy of 10 years (patient of Dr. C. C. Forsyth).

FIG. 5.—Acanthocytes in α - β -lipoproteinaemia. Stained dried blood film (left) and fresh wet preparation of whole blood (right).

estimation of the individual phospholipids shows a relative decrease in phosphatidyl choline and increase in sphingomyelin; the amount of linoleic acid in phosphatidyl choline is greatly reduced (Jones and Ways, 1967). The total concentration of NEFA is normal (Salt *et al.*, 1960). Vitamin E and carotenoid pigments which are transported by β -lipoprotein are absent from the serum, and vitamin A levels are low.

Acanthocytosis of the red blood cells is always present, but in a dried blood film may be mistaken for crenation, and an undiluted fresh wet preparation should always be examined (Fig. 5). Normal rouleaux formation is absent and the sedimentation rate is abnormally low. Osmotic fragility is normal or only slightly decreased. Autohaemolysis at 37° C. and 4° C. is greatly increased (Simon and Ways, 1964), as is peroxidative haemolysis (Dodge *et al.*, 1967). The concentrations of total cholesterol and phospholipid in the red cell membrane are normal, but the distribution of phospholipid is abnormal, with an increase in sphingomyelin and decrease in phosphatidyl choline. This abnormality is probably secondary to exposure of the cells to the abnormal plasma (Ways and Simon, 1964).

The steatorrhoea is due to failure of chylomicron formation; the concentrations of bile acids and pancreatic enzymes in the bowel are normal. Intestinal biopsy shows a normal villous pattern, but the columnar cells are distended with lipid (Fig. 6) which is shown on chemical analysis to be largely triglyceride (Ways *et al.*, 1967). Despite this block in fat absorption a considerable amount (at least 80%) of dietary fat is absorbed, and the fatty acids are presumably transported via the portal blood stream (Kayden and Medick, 1967). The reason for the failure of chylomicron formation in α - β -lipoproteinaemia is not clear. Triglyceride

also accumulates in the liver in this condition (Isselbacher *et al.*, 1964; Bach *et al.*, 1967), and it is suggested that β -lipoprotein may have a specific function in the transport of both exogenous and endogenous triglyceride from cells (Fredrickson *et al.*, 1967).

Diagnosis. The serum cholesterol should be estimated in all cases of steatorrhoea, of retinitis pigmentosa, or of a Friedreich-like ataxia; levels below 60 mg./100 ml. make the diagnosis likely, and β -lipoprotein should then be estimated by one of the methods already mentioned. The finding of acanthocytes is not pathognomonic, as they have also been found in patients with hepatic disease (Smith, Lonergan, and Sterling, 1964); neither is the appearance of the intestinal mucosa diagnostic, as Anderson *et al.* (1961) have described appearances identical with those in α - β -lipoproteinaemia in a patient whose blood had no acanthocytes and only moderately reduced levels of β -lipoprotein.

It has recently become apparent that familial deficiency, as distinct from absence, of β -lipoprotein occurs, and there probably exists a spectrum of conditions with varying degrees of β -lipoprotein deficiency (Kuo and Basset, 1962; Tolentino, Spirito, and Jannuzzi, 1964; Van Buchem *et al.*, 1966).

Treatment. Steatorrhoea can be abolished by a low fat diet (usually less than 20 g. fat daily) and on such a regimen normal growth may be achieved (Fig. 7). In babies and others in whom calorie intake on a low fat diet may be insufficient, medium chain triglycerides, which are absorbed directly into the portal blood stream and do not enter into chylomicron formation, may be used (Fig. 8). Because of the low levels of linoleic acid in the plasma and red cell membrane, some of the dietary

FIG. 6.—*Jejunal mucosa in α - β -lipoproteinaemia. Showing accumulation of lipid (stained with Sudan Black B) in epithelial cells (Dr. C. Berry).*

fat should be polyunsaturated. Large doses of a water miscible preparation of vitamin A (20,000 units daily) are required to achieve normal plasma levels, and additional vitamin K and D should also be given. Autohaemolysis of the red cells can be restored to normal or near normal by the oral administration of a water miscible preparation of vitamin E (1.5 g. DL- α -tocopherol acetate daily), but peroxidative haemolysis is not so readily corrected (Dodge *et al.*, 1967), and normal serum levels of the vitamin cannot be achieved, possibly because of lack of the carrier protein.

Until the pathogenesis of the retinopathy and neuropathy is understood, therapeutic and preventive measures can only be empirical. The maintenance of normal plasma vitamin A levels from the age of 2 years did not prevent the development of retinopathy in one patient at the age of 5 years (Wolff *et al.*, 1964), though subsequently there has been no deterioration in visual appearance or function in this child. The possible role of vitamin E in maintaining integrity of nerve cell membranes has not yet been elucidated.

Familial α -lipoprotein deficiency (Tangier disease). Familial α -lipoprotein deficiency is

associated with storage of cholesterol esters in many parts of the body, particularly in the reticuloendothelial system, and was named Tangier disease after the Chesapeake Bay island home of the first patients, a 5-year-old boy and his 6-year-old sister (Fredrickson *et al.*, 1961). Since this time a total of 9 cases has been reported.

Genetics. The disease is inherited as an autosomal recessive; the primary gene effect is probably concerned with the production of a defective protein. The heterozygotes have no relevant clinical abnormalities, but have reduced concentrations of α -lipoprotein in the serum and may have fasting hypertriglyceridaemia (Fredrickson, 1966). The α -lipoprotein levels in the women are particularly labile and it may be difficult to identify the female heterozygotes with certainty.

Clinical features and laboratory findings. The outstanding and diagnostic clinical feature is gross enlargement of the tonsils which are yellow-orange in colour due to deposition of cholesterol esters. Even if the tonsils have been removed the distinctive appearance can still be recognized in the lymphoid tags in the tonsillar fossae and in the plaques of

FIG. 7.—Treatment of α - β -lipoproteinaemia. Growth response to low-fat diet.

FIG. 8.—Treatment of α - β -lipoproteinaemia. Growth response to a diet low in ordinary fat (LCT) and supplemented with medium chain triglycerides (MCT).

pharyngeal lymphoid tissue. Lymphadenopathy, hepatomegaly, and splenomegaly may occur during childhood, and in adolescence and adult life peripheral neuropathy (Kocen *et al.*, 1967; Engel *et al.*, 1967) and corneal infiltration (Hoffman and Fredrickson, 1965) have been reported. Deposition of cholesterol ester has also been demonstrated in the bone-marrow and rectal mucous membrane.

The serum is characteristically turbid in the fasting state, and triglyceride levels are moderately raised (200–300 mg./100 ml.). Concentrations of total cholesterol are reduced to about 70 mg./100 ml. and phospholipids to about 100 mg./100 ml. The distribution of the individual phospholipids shows an increase in the percentage of phosphatidyl choline and decrease in sphingomyelin, which is directly opposite to the abnormality found in α - β -lipoproteinaemia. On paper electrophoresis no α -lipoprotein can be detected and the β -lipoprotein band has the advanced mobility of pre- β -lipoprotein (Kocen *et al.*, 1967). Analysis after ultracentrifugation shows that most of the lipid is of very low density (<1.019 , corresponding to pre- β -lipoprotein), and that there is an excess of triglyceride in all lipoprotein fractions. A small amount of α -lipoprotein can be demonstrated by this technique and has been shown by immunochemical studies (Levy and Fredrickson, 1966) to be an abnormal protein; it has been designated as α 'T' lipoprotein. The fatty acid composition of the individual lipids is normal.

The red blood cells are normal morphologically, and the membrane contains normal concentrations of total cholesterol and phospholipids, but there is a relative increase of phosphatidyl choline and decrease in sphingomyelin (M. M. Shacklady and J. K. Lloyd, unpublished). Autohaemolysis and plasma vitamin E levels are normal.

Treatment and prognosis. In children the condition does not appear to give rise to disability but in some young adults a neuropathy has developed, and one patient died at the age of 48 years, probably from myocardial infarction. The mechanisms responsible for the accumulation of cholesterol in the cells and triglyceride in the serum are not understood, and no attempts have yet been made to influence the former. Serum triglyceride concentrations vary markedly in response to carbohydrate feeding (Levy *et al.*, 1966), and the carbohydrate intake of these patients should probably be restricted.

Secondary Deficiencies of Serum Lipoproteins

Chylomicrons. These are normally only found during fat absorption. The failure to form chylomicrons after a fat-containing meal in α - β -lipoproteinaemia has already been described; a reduction in the chylomicron concentration occurs in any condition in which fat absorption is impaired, and is especially marked when intraluminal emulsification is defective as in cystic fibrosis of the pancreas. This reduction in serum lactescence after a fatty meal has been used as a diagnostic test in the investigation of fat absorption (Hadorn *et al.*, 1966). Unfortunately, factors such as the rate of gastric emptying and the rate of plasma clearance make interpretation difficult, but the test is of some value in circumstances where other methods of estimating fat absorption cannot be used.

β -lipoprotein. Reduction in the concentration of β -lipoprotein occurs when synthesis is impaired as in hepato-cellular failure and malabsorptive states, when katabolism is increased as in hyperthyroidism, or when there is increased loss as in protein-losing enteropathy. Caution is needed in interpreting the paper electrophoretic pattern in hepato-cellular failure; in these circumstances cholesterol esterification may be impaired, and reduction in the β -lipoprotein band on the paper strip can be due to the fact that unesterified cholesterol does not take up the lipid stain. Estimations of total serum cholesterol should always be made in addition to electrophoresis.

Hypolipidaemia with reduction in both serum cholesterol and phospholipid levels has been described in association with chronic anaemia due to a wide variety of causes (Rifkind and Gale, 1967), and reduction in β -lipoprotein has been found in patients with the thalassaemia trait (Fessas, Stamatoyannopoulos, and Keys, 1963). The mechanisms responsible are not understood. Low levels of β -lipoprotein are also found in familial hyperchylomicronaemia (fat-induced hypertriglyceridaemia).

α -lipoprotein. It has been shown (Levy *et al.*, 1966) that α -lipoprotein is necessary for the formation of pre- β -lipoprotein and tends to be reduced whenever the concentration of pre- β -lipoprotein is increased. Low levels of α -lipoprotein are also found in many acute and chronic illnesses in childhood, and gross reduction may occur in patients with biliary obstruction.

REFERENCES

- Anderson, C. M., Townley, R. R. W., Freeman, M., and Johansen, P. (1961). Unusual causes of steatorrhoea in infancy and childhood. *Med. J. Aust.*, **2**, 617.
- Auerswald, W., Doleschel, W., and Müller-Hartburg, W. (1963). Flotationsanalytische Untersuchungen der Lipoproteinverteilung im mütterlichen und im Nabelschnurblut. *Klin. Wschr.*, **41**, 580.

- Bach, C., Polonovski, J., Polonovski, C., Leluc, R., Jolly, G., and Moszer, M. (1967). L'absence congénitale de beta-lipoprotéines. *Arch. franç. Pédiat.*, **24**, 1093.
- Crowley, J., Ways, P., and Jones, J. W. (1965). Human fetal erythrocyte and plasma lipids. *J. clin. Invest.*, **44**, 989.
- Dodge, J. T., Cohen, G., Kayden, H. J., and Phillips, G. B. (1967). Peroxidative hemolysis of red blood cells from patients with abetalipoproteinemia (acanthocytosis). *ibid.*, **46**, 357.
- Engel, W. K., Dorman, J. D., Levy, R. I., and Fredrickson, D. S. (1967). Neuropathy in Tangier disease. *Arch. Neurol. (Chic.)*, **17**, 1.
- Fessas, P., Stamatiyannopoulos, G., and Keys, A. (1963). Serum-cholesterol and thalassaemia trait. *Lancet*, **1**, 1182.
- Fredrickson, D. S. (1966). Tangier disease. In *The Metabolic Basis of Inherited Disease*, 2nd ed., p. 486. Ed. by J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson. McGraw-Hill, New York.
- , Altrocchi, P. H., Avioli, L. V., Goodman, De W. S., and Goodman, H. C. (1961). Tangier disease. *Ann. intern. Med.*, **55**, 1016.
- , Levy, R. I., and Lees, R. S. (1967). Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *New Engl. J. Med.*, **276**, 32.
- Hadorn, B., Zoppi, G., Launiala, K., Shmerling, D. H., and Prader, A. (1966). Ein einfacher, orientierender Test zur Beurteilung der Fettersorption bei Kindern: die Messung der Serumtrübung nach Butterfetteinnahme. *Helv. paediat. Acta*, **21**, 387.
- Hoffman, H. N., and Fredrickson, D. S. (1965). Tangier disease: (familial high density lipoprotein deficiency): clinical and genetic features in two adults. *Amer. J. Med.*, **39**, 582.
- Isselbacher, K. J., Scheig, R., Plotkin, G. R., and Caulfield, J. B. (1964). Congenital beta-lipoprotein deficiency: an hereditary disorder involving a defect in the absorption and transport of lipids. *Medicine (Baltimore)*, **43**, 347.
- Jones, J. W., and Ways, P. (1967). Abnormalities of high density lipoproteins in abetalipoproteinemia. *J. clin. Invest.*, **46**, 1151.
- Kayden, H. J., and Medick, M. (1967). Alternative pathways of long chain fatty acid absorption in the absence of chylomicron formation. *ibid.*, **46**, 1077.
- Kocen, R. S., Lloyd, J. K., Lascelles, P. T., Fosbrooke, A. S., and Williams, D. (1967). Familial α -lipoprotein deficiency (Tangier disease) with neurological abnormalities. *Lancet*, **1**, 1341.
- Kuo, P. T., and Basset, D. R. (1962). Blood and tissue lipids in a family with hypo-beta-lipoproteinemia. *Circulation*, **26**, 660.
- Lees, R. S. (1967). Immunological evidence for the presence of B protein (apoprotein of β -lipoprotein) in normal and abetalipoproteinemic plasma. *J. Lipid Res.*, **8**, 396.
- Levy, R. I., and Fredrickson, D. S. (1965). Heterogeneity of plasma high density lipoproteins. *J. clin. Invest.*, **44**, 426.
- , and — (1966). Nature of the alpha lipoproteins in Tangier disease. *Circulation*, **33-34**, Suppl. 3, 156.
- , Lees, R. S., and Fredrickson, D. S. (1966). The nature of pre-beta (very low density) lipoproteins. *J. clin. Invest.*, **45**, 63.
- Lindquist, B., and Malmcrona, R. (1960). Dietary fat in relation to serum lipids in the normal infant. *Amer. J. Dis. Child.*, **99**, 39.
- Margolis, S., and Langdon, R. G. (1966). Studies on human serum B₁-lipoprotein. I. Amino acid composition. *J. biol. Chem.*, **241**, 469.
- Mortimer, J. G. (1964). Cord blood lipids of normal infants and infants of diabetic mothers. *Arch. Dis. Childh.*, **39**, 342.
- Persson, B., and Gentz, J. (1966). The pattern of blood lipids, glycerol and ketone bodies during the neonatal period, infancy and childhood. *Acta paediat. (Uppsala)*, **55**, 353.
- Phillips, G. B., and Dodge, J. T. (1967). Composition of phospholipids and of phospholipid fatty acids of human plasma. *J. Lipid Res.*, **8**, 676.
- Rafstedt, S. (1955). Studies on serum lipids and lipoproteins in infancy and childhood. *Acta paediat. (Uppsala)*, **44**, Suppl. 102.
- Rey, J. (1961). *L'Absence Congénitale de Beta-lipoprotéines*. R. Foulon, Paris.
- Rifkind, B. M., and Gale, M. (1967). Hypolipidaemia in anaemia: implications for the epidemiology of ischaemic heart-disease. *Lancet*, **2**, 640.
- Salt, H. B., and Wolff, O. H. (1957). The applications of serum lipoprotein electrophoresis in paediatric practice. *Arch. Dis. Childh.*, **32**, 404.
- , —, Lloyd, J. K., Fosbrooke, A. S., Cameron, A. H., and Hubble, D. V. (1960). On having no beta-lipoprotein: a syndrome comprising a-beta-lipoproteinemia, acanthocytosis, and steatorrhea. *Lancet*, **2**, 325.
- Scanu, A. M. (1965). Factors affecting lipoprotein metabolism. *Advanc. Lipid Res.*, **3**, 63.
- Simon, E. R., and Ways, P. (1964). Incubation hemolysis and red cell metabolism in acanthocytosis. *J. clin. Invest.*, **43**, 1311.
- Smith, J. A., Lonergan, E. T., and Sterling, K. (1964). Spur-cell anaemia: hemolytic anaemia with red cells resembling acanthocytes in alcoholic cirrhosis. *New Engl. J. Med.*, **271**, 396.
- Sobrevilla, L. A., Goodman, M. L., and Kane, C. A. (1964). Demyelinating central nervous system disease, macular atrophy and acanthocytosis (Bassen-Kornzweig syndrome). *Amer. J. Med.*, **37**, 821.
- Sweeney, M. J., Etteldorf, J. N., Orban, P. M., and Fischer, R. (1962). Effect of diet during first six to eight weeks of life on total concentrations of serum protein and on electrophoretic patterns of serum protein and lipoprotein. *Pediatrics*, **29**, 82.
- Tolentino, P., Spirito, L., and Jannuzzi, C. (1964). Celiac syndrome, with retinal dystrophy and acanthocytosis, without defect of beta lipoprotein. *Ann. paediat. (Basel)*, **203**, 178.
- Van Buchem, F. S. P., Pol, G., de Geir, J., Böttcher, C. J. F., and Pries, C. (1966). Congenital beta-lipoprotein deficiency. *Amer. J. Med.*, **40**, 794.
- Ways, P. O., Parmentier, C. M., Kayden, H. J., Jones, J. W., Saunders, D. R., and Rubin, C. E. (1967). Studies on the absorptive defect for triglyceride in abetalipoproteinemia. *J. clin. Invest.*, **46**, 35.
- , and Simon, E. R. (1964). The role of serum in acanthocyte autohemolysis and membrane lipid composition. *ibid.*, **43**, 1322.
- Wolff, O. H., Lloyd, J. K., and Tonks, E. L. (1964). A-beta-lipoproteinemia with special reference to the visual defect. *Exp. Eye Res.*, **3**, 439.

APPENDIX

ANALYTICAL METHODS

The following notes are intended as a guide to the collection and handling of samples and give the methods used in our laboratory.

Collection of Blood

In order to interpret the results of investigations on serum lipids and lipoproteins it is essential that blood is obtained in the fasting state unless special tests of the lipoprotein response to a single load of fat or carbohydrate are being conducted. It is also useful to know the dietary history of the individual in the preceding few

weeks. For babies the period of fasting should be 8 hours and for older children 12–16 hours; only water is allowed during this interval. Lipoproteins, especially chylomicrons and pre- β -lipoproteins, deteriorate on storing and the pattern is altered irreversibly by deep freezing. Serum lipoprotein fractionation by electrophoresis or ultracentrifugation should therefore be carried out within 24 hours of collection. The use of serum is preferred to plasma for analysis, as the addition of anticoagulants (especially heparin) distorts the electrophoretic pattern, and also changes the red cell/

plasma volume ratio. When plasma is required for a special reason such as the simultaneous analysis of the red cell membrane lipids, the most suitable anticoagulant is sodium ethylene diaminetetra-acetate (EDTA), 1 mg./ml. blood.

Electrophoresis

Paper electrophoresis is a simple reliable technique which can be performed in any laboratory. We use the method of Salt and Wolff (1957) with minor modifications. Electrophoresis is carried out on 2.5 cm. width strips of Whatman 3 mm. paper in horizontal tanks (Shandon) using 0.06 M barbitone buffer at pH 8.6 and a current of approximately 0.5 mA per strip over a period of 16 hours. A serum volume of 0.075 ml. is loaded for lipoprotein separation, and 0.01 ml. of the same sample is loaded on an adjacent strip for subsequent staining for serum proteins. The lipoprotein strips are stained with oil Red O and the protein strips with bromophenol blue. Pre- β -lipoprotein is not completely separated from β -lipoprotein by this technique, but the forward mobility is easily appreciated by alignment with the protein 'marker' strip, when the pre- β -lipoprotein will be found to occupy the zone between β - and α_2 -globulin.

The method described by Fredrickson, Levy, and Lees (1967) uses EDTA plasma for separation, barbitone buffer of ionic strength 0.1 and pH 8.6, containing 1% albumin and 0.001 M EDTA, and a vertical (hanging-strip) Durrum apparatus. We have not found the incorporation of albumin in the buffer to give better separation in our method, and consider the absence of protein marker strips a disadvantage.

Quantification of lipoproteins by elution of dye from the paper strips requires a larger serum load (0.15 ml.) on a wider strip (5.0 cm.), and is of limited value since dye uptake by the individual lipid classes varies, being greatest for triglycerides and cholesterol esters, and considerably less for phospholipids; thus, β -lipoprotein tends to be overestimated and α -lipoprotein underestimated. Quantification is therefore probably best carried out by ultra-centrifugal or immunochemical methods.

Ultracentrifugation. In the preparative ultracentrifuge we have used the salt-density gradient method of Cornwell *et al.* (1961). Chylomicrons, if present, are removed by a preliminary spin at 10,000 $\times$ g for 30 minutes. This method requires about 4-5 ml. serum, is time consuming, needs expensive apparatus, and is not suitable for routine analysis.

Immunological techniques. By the use of specific antisera the protein moiety of the lipoproteins can be studied. Immuno-electrophoresis has been carried out by the method of Grabar and Williams (1955) and

semiquantitative determinations of β - and α -lipoproteins by the Ouchterlony technique (Gell, 1957).

Chemical analysis of individual lipids. Lipids have been extracted from the serum at room temperature overnight in ethanol/ether 3 : 1, using a 1 in 20 dilution. Individual lipids have been analysed by the following methods. Total cholesterol by the method of Brown (1961) using the Lieberman-Burchard reaction, and more recently by the auto analyser using the method (N24a) described by Technicon*; total phospholipids (lipid phosphorus $\times$ 25) and individual phospholipids (separated by thin-layer chromatography) by the method of Bartlett (1959); triglycerides by the method of Freeman (1964) using infrared absorptiometry, or by gas-liquid chromatography using an internal standard (Fosbrooke and Tamir, 1968); non-esterified fatty acids by the Dole titration method (Dole and Meinertz, 1960) or by gas-liquid chromatography using an internal standard (Fosbrooke and Tamir, 1968).

The fatty acid pattern of the individual lipids has been determined by gas-liquid chromatography of the methyl esters prepared by transmethylation with methanol/sulphuric acid after preliminary separation by thin-layer chromatography. If any lipid fraction is suspected of containing the medium chain fatty acids C8 : 0 and C10 : 0 (octanoic and decanoic) special precautions are necessary owing to their water solubility and the volatility of their methyl esters.

REFERENCES

- Bartlett, G. R. (1959). Phosphorus assay in column chromatography. *J. biol. Chem.*, **234**, 466.
- Brown, W. D. (1961). A filtration procedure for determining serum cholesterol with digitonin. *Aust. J. exp. Biol. med. Sci.*, **39**, 223.
- Cornwell, D. G., Kruger, F. A., Hamwi, G. J., and Brown, J. B. (1961). Studies on the characterization of human serum lipoproteins separated by ultracentrifugation in a density gradient. *Amer. J. clin. Nutr.*, **9**, 24.
- Dole, V. P., and Meinertz, H. (1960). Microdetermination of long-chain fatty acids in plasma and tissues. *J. biol. Chem.*, **235**, 2595.
- Fosbrooke, A. S., and Tamir, I. (1968). A modified method for the preparation of methyl esters of a mixture of medium-chain and long-chain fatty acids. *Clin. chim. Acta*. In the press.
- Fredrickson, D. S., Levy, R. I., and Lees, R. S. (1967). Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. *New Engl. J. Med.*, **278**, 94.
- Freeman, N. K. (1964). Simultaneous determination of triglycerides and cholesterol esters in serum by infrared spectrophotometry. *J. Lipid Res.*, **5**, 236.
- Gell, P. G. H. (1957). The estimation of the individual human serum proteins by an immunological method. *J. clin. Path.*, **10**, 67.
- Grabar, P., and Williams, C. A., Jr. (1955). Méthode immuno-electrophorétique d'analyse de mélanges de substances antigéniques. *Biochim. biophys. Acta (Amst.)*, **17**, 67.
- Salt, H. B., and Wolff, O. H. (1957). The applications of serum lipoprotein electrophoresis in paediatric practice. *Arch. Dis. Childh.*, **32**, 404.

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