ASSESSMENT OF A LABORATORY METHOD FOR THE EVALUATION OF HYPERLIPOPROTEINAEMIA

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Introduction

It is now generally accepted that hyperlipidaemia must be interpreted in terms of hyperlipoproteinaemia, for it is as lipoproteins that the major plasma lipids circulate, and any quantitative change in one or more of the lipids reflects a disturbance in the lipoproteins. (Lancet, 1972). This view underlies the re-examination of the hyperlipidaemias lately undertaken in order to classify them in terms of lipoprotein alterations and has given rise to several alternative classifications, of which that from the National Institutes of Health has gained the widest acceptance. This has been endorsed with a slight modification by the W.H.O. (Beaumnet 1970).

Strisower *et al.* (1968) introduced another classification that is complementary in many respects to that of the N.I.H. Such differences that arise may be due to the fact that Fredrickson concentrated on familial hyperlipoproteinaemia while Strisower studied the lipid profile of the general population.

The W.H.O. Memorandum sets out, in detail, diagnostic criteria and recommended laboratory methods. It emphasizes that any current classification, while clinically useful, is necessarily open-ended and incomplete, and awaits a definition of the precise aetiology of each of these

Table I

Classification of Hyperlipoproteinaemia (3)

Type	*I*	*IIa*	*IIb*	*III*	*IV*	*V*
Laboratory findings						
Chylomicrons	+++	normal	normal	normal	normal	++
β-lipoproteins	normal	+++	+++	normal	normal	normal
Pre-β-lipoproteins	normal	normal	+	++ (subgroup)	+++	++
Plasma turbidity	+++	−	(+)	(++)	(+++)	+++
Triglycerides	+++	normal	+	++	++	+++
Cholesterol	(+)	+++	+++	++	++	(+)
Glucose tolerance	normal	normal	(↓)	(↓)	↓	(↓)
Lipoprotein lipase	↓	−	−	−	−	(↓)
Clinical findings						
Carbohydrate-induced	−	−	(+)	(+)	++	(+)
Fat-induced	+++	−	−	−	−	++
Xanthomas	eruptive	tendon tuberous	tendon tuberous	palmar tuberous	eruptive	eruptive
Xanthelasmas	−	+	+	+	−	−
Hepatosplenomegaly	++	−	−	−	+	+
Pancreatitis	++	−	−	−	+	++
Arteriosclerosis	?	+++	+++	++	++	+

Table II

Lipoprotein constituents (II)

	High density lipoproteins (alpha)	*Low density lipoproteins (beta)*	*Very low density lipoproteins (pre-beta)*	*Chylomicrons*
Density (g/ml)	1.210-1.063	1.063–1.006	1.006	1.006
S f		0–2	20–400	400
Particle size	70–100 A	100–300 A	2,000 A	2,000 A
Protein	49%	32%	2–13%	½%
Triglycerides	7%	7%	64–80%	90%
Cholesterol	17%	35%	8–13%	6%
Phospholipids	27%	25%	6–15%	4%
Cholesterol/phospholipids ratio	0.6	1.4	1.4	1.5

disorders. The W.H.O. Classification (Table I) is compatible with any system which reliably identifies and measures the individual lipoproteins and recognizes certain normal and abnormal variations.

Plasma lipoproteins differ in their densities, flotation rates, electrophoretic mobility, relative content of triglycerides, cholesterol and phospholipid and in the types and content of apo-protein. (Table II) Accordingly they may be separated by ultracentrifugation, precipitation or electrophoresis.

For primary screening the last technique is the method of choice; polyacrylamide gel, paper or cellulose acetate membrane media (C.A.M.) may be used. Four or more distinct bands may be identified after appropriate staining. Different patterns of these bands (phenotypes) are observed in different groups of individuals.

Methods

The subjects were classified according to the W.H.O. Classification on the basis of the biochemical findings resulting from the applied techniques, without consideration of clinical or genetic information. Samples derived from patients who had recently suffered a myocardial infarction were excluded from the present study.

Electrophoresis was performed on C.A.M. as described by Charman and Landowne, 1967 and on paper as described by Fredrickson, Levy and Lees (1967). Lipoproteins were stained with the Ozone-Schiff method as described by Kohn (1961) and with the Oil-Red-O technique as described by Winkelman *et al* (1969).

Serum proteins were stained with Ponceau. The reporting system of Winkelman and Ibbot (1969) was adopted. Visual interpretation of electrophoretograms was re-assessed at an interval of 1 year. The technical advantages of the two techniques were assessed.

Serum cholesterol and β-lipoproteins were estimated by the acetic anhydride method and triglycerides by an enzymatic method using Boehringer kits.

Results

The samples analyzed by paper electrophoresis could not be definitely evaluated. In particular, however, paper electrophoresis consistently failed to separate the β- and pre-β bands. This finding is consistent with previous studies (Winkelman & Ibbot 1969).

Of 24 samples that were evaluated by C.A.M. electrophoresis and stained with the Ozone-Schiff method, 4 were unclassifiable and 3 were non-definitive IIa or IIb. Seventeen could be definitely phenotyped and of these 8 were II a and 9 were II b.

Another set of 24 samples were analysed by C.A.M. electrophoresis and stained with the Oil-Red-O method, and by biochemical lipid profile. Two of these samples could not be definitely classified. Twelve could be definitely classified as type II a, of which 2 did not agree with the biochemical data; 10 could be definitely classified as type II b of which 2 did not agree with the biochemical data. In 18 out of 24, visual interpretation of the lipoprotein electrophoretogram was in agreement with the biochemical lipid profile.

β-Lipoprotein was estimated in 16 samples with a raised serum cholesterol of which 8 had a β-lipoprotein level of 600 mg % or above representing the range from a high normal to an abnormally high level. All 8 were type II b phenotypes.

The Ozone-Schiff technique enabled the classification of 17 of the 24 samples, while using Oil-Red-O staining 18 samples of the 24 in the series could be definitely classified.

After an interval of one year all electrophoretograms stained with the Ozone-Schiff method could easily be read and there was agreement with the original interpretation. The bands stained with Oil-Red-O could only be re-developed with difficulty after immersing in glycerine, and the bands were indistinct even then, with the result that no interpretation was possible.

Conclusion

It is concluded that cellulose acetate membrane electrophoresis followed by Ozone-Schiff staining of the lipoprotein electrophoretogram and biochemical lipid profiling is a simple and reliable method for phenotypng hyperlipoproteinaemia. It permits a precise diagnosis to be made in a large number of cases.

Moreover, Ozone-Schiff staining produces electrophoretograms that are permanent and easily re-interpreted at any time and in conjunction with any new information that becomes available. Because of this we consider it superior to the Oil-Red-O technique.

The laboratory evaluation of patients with elevated plasma lipids is of increasing importance in view of consistent reports associating hyperlipidaemia particularly in middle-aged or younger population groups with increasing risk to ischaemic heart disease.

The lipoprotein phenotype has also been shown to be relevant to the choice of treatment to be followed as in many instances, dietary measures have to be supplemented by drugs, principally Clofibrate which reduces primarily the Very Low Density Lipoprotein (Humminghoke *et al* 1969) and Cholestyramine which reduces primarily the Low Density Lipoprotein (Fallon & Woods, 1968).

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References

BEAUMNET *et al.* (1970). Bull. W.H.O. 4th Org. *43,* 491.

CHARMAN, R.C. and LANDOWNE, R.A. (1967). Anal. Biochem, *19,* 177.

FALLON, H.J. & WOODS, J.W. (1968). J. Amer. Med. Ass. *204,* 1161.

FURMAN *et al.* (1966). Pathophysiological and Clinical Aspects of lipid metabolism,

FREDRICKSON *et al.* (1967). New Engl. J. of Med. *276,* 32, 94,, 143, 215, 273.

HUMMINGHOKE *et al.* (1969). Circulation, *39,* 675.

KOHN, J. (1961). Nature, *189*, 312.
Leading Article, Lancet. (1971). *1*. 276.
LEHMAN & LINES. (1972). Lancet, *1*, 557.
STRISOWER *et al.* (1968). Amer. J. Med. *45*, 488.
WINKELMAN *et al.* (1969). Clin. Chim. Acta *26*, 33.
WINKELMAN, J. & OBBOTT, F.A. (1969). Clin. Chim. Acta *26*, (25).