THE APPLICATIONS OF SERUM LIPOPROTEIN ELECTROPHORESIS IN PAEDIATRIC PRACTICE

BY

H. B. SALT and O. H. WOLFF

From the Children's Hospital and University Department of Paediatrics, Birmingham

(RECEIVED FOR PUBLICATION APRIL 5, 1957)

In the investigation of many metabolic disorders of childhood, a study of the serum lipids and lipoproteins is important. Serum protein electrophoresis has become an everyday tool in most laboratories; lipoprotein electrophoresis has not yet attained such acceptance, though its research value is becoming recognized.

Studnitz (1955) studied the normal lipoprotein values in pregnancy and Rafstedt (1955) those in infancy and childhood. Other recent publications include surveys of lipoprotein investigations in various diseases that involve lipid metabolism (Adlersberg, Bossak, Sher and Sobotka, 1955; Dangerfield and Smith, 1955) and detailed observations in idiopathic hyperlipaemia and hypercholesterolaemia (Herbst, Lever and Hurley, 1955), atherosclerosis (Gottfried, Pope, Friedman, Akerson and Di Mauro, 1955) and malnutrition (Laurell, 1955).

We have applied a method of lipoprotein electrophoresis to some conditions encountered in paediatric hospital practice and describe here a selection from our results. We have found the method of value in various diseases which involve lipid metabolism both primarily and secondarily.

The main types of serum lipid, apart from minute concentrations of steroids and other compounds of undoubted significance but not to be discussed here, are triglyceride esters usually present as discrete particles, cholesterol and cholesteryl esters held in solution by physical bonding to one or more of the serum proteins, and phospholipids similarly bound to serum proteins.

The process of electrophoretic separation of serum proteins on absorbent paper is readily adapted to the separation of three fractions of serum lipid. Examination of the fractions (Kunkel and Slater, 1952) has shown that alpha lipoprotein consists of alpha globulin combined with phospholipid (66% of the total alpha lipid) and with cholesterol and its esters (33% of the total alpha lipid); and that beta lipoprotein consists of beta

globulin combined with phospholipid (44% of the total beta lipid) and with cholesterol and its esters (56% of the total beta lipid). A third component, which we have designated the omega fraction, consists of triglyceride esters in nearly pure state in the form of chylomicron particles. The protein-bound lipids migrate electrophoretically with speeds approximating to those of the globulins concerned, though with certain subtle differences to be discussed later; whereas the omega fraction, because of adsorption to the paper, appears devoid of electrophoretic mobility and remains at or close to the origin of the electrophoretic separation.

In the present study of serum proteins and lipoproteins we have confined ourselves to electrophoretic separations, though of course that technique does not distinguish between the various types of lipid material in the several individual fractions.

Our technique is based on procedures described by Swahn (1952a, 1952b) and by Adlersberg *et al.* (1955), and is described here in detail as we have developed certain modifications; it includes a quantitative assessment of the serum total lipid and its fractions.

METHODS

Venous blood, collected with a silicone- (not paraffin) coated syringe, was allowed to clot and the serum removed after centrifuging for only 5 to 10 minutes at 2,500 r.p.m. to avoid separation of low specific gravity lipids. A minute drop of 10% sodium azide was added as preservative and the serum stored at 4° C. Electrophoresis was performed on the specimens within a few days of collection, as occasionally prolonged storage was found to cause artefacts in the electrophoretic lipid patterns.

The electrophoretic separations were done in a horizontal apparatus similar to that of Grassmann, Hannig and Knedel (1951), using Whatman No. 3 mm. paper and 0.06 M. barbitone buffer at pH 8.6. Each paper strip, 13.5 × 2.0 in. (12 in. between the buffer solutions in the reservoirs), was marked in pencil transversely at a point 5 in. from the cathode end; and six such papers when arranged in parallel were conveniently

operated by a current of 12 mA. during an overnight period of about 16 hours.

Before application of the serum, the paper strip was soaked in buffer solution and then firmly blotted. A minute drop of a saturated solution of bromophenol blue (B.D.H. water-soluble indicator) was added to the serum; a volume of 0.15 ml. coloured serum was then pipetted on to the pencilled origin line. A duplicate paper strip was also prepared, and a third in a similar manner but using only 0.05 ml. serum. When a further set of three papers was also ready, all six were placed in the electrophoresis apparatus, and drops of buffer solution were then pipetted asymmetrically on to the ends of the papers to achieve saturation equilibrium. By judicious placing of the drops the serum load was prevented from moving from the origin line. The apparatus was then closed. After waiting 30 minutes or more for vapour equilibrium to become established, the current was applied.

When the albumin-bound bromophenol blue had migrated about 4 in. toward the anode, the electrophoretic separation was discontinued; the papers were removed and dried for 30 minutes at 100° C. One paper, loaded with 0.15 ml. serum, was then prepared for quantitative determination of the separated lipids. To the centre of an unoccupied area of the paper strip near the cathode end (measuring 1½ in. in length) a standard quantity (0.37 mg.) of arachis oil was applied in the form of 0.08 ml. of a solution containing 0.5 ml. arachis oil per 100 ml. solvent (ether-ethanol 1:1). When the solvent had evaporated, the serum lipids and the standard spot were stained by overnight immersion of the paper strip in a freshly prepared saturated solution of oil red O (George T. Gurr, Ltd.) in ethanol-water (60:40). The paper was then washed, first in ethanol-water (60:40) for about half a minute, then in water for about half an hour, after which it was hung in the air until dry.

The paper was then cut transversely into ⅜ in.-wide segments, of which five comprised the standard spot and 20 the serum lipids including one or more blank segments. Each segment was then placed in a test tube and 6.0 ml. propanolacetic acid mixture (80:20) added to each tube. After standing overnight the concentration of eluted oil red O dye in each of the tubes was determined absorptiometrically using narrow-band light at 525 millimicrons peak wave length.

The extinction values were then plotted as ordinates against linear distance and the serum lipid profile completed (Fig. 1A). The graph was then subdivided into three parts representing the omega fraction and the

beta and alpha lipid peaks. Demarcation of the omega fraction from the beta peak was difficult in some cases. Particularly large omega fractions, or their absence, were obvious, but intermediate values sometimes presented merely as a convexity on the cathode slope of the beta peak. In such cases the component curves were extrapolated and the contributory extinction values were carefully allocated to effect a subdivision.

Grouped extinction values were summed to represent the three fractions, and the amounts of the fractions were then calculated by reference to the extinction sum for the standard spot. The resulting values for the alpha, beta and omega lipid fractions and total lipid were thus obtained in terms of their uptake of oil red O relative to the uptake by arachis oil.

The other two electrophoretic paper strips (0.15 and 0.05 ml. serum) were each divided longitudinally; one-half of each was stained with oil red O, and the other half with bromophenol blue to reveal albumin and alpha₁, alpha₂, beta and gamma globulins, using the aqueous staining reagent and subsequent acid-aqueous washing technique described by Kunkel and Tiselius (1951).

A careful comparison of these stained papers yielded qualitative information about the patterns and the mobility correspondence between the serum lipid fractions and the protein fractions. Qualitative features of the protein pattern were clearer on the lightly loaded paper, and of the lipid pattern on the more heavily loaded paper. As a rule the mobility correspondence of the beta lipoprotein peak was assessed from inspection of the lightly loaded paper, whereas the properties of the more faintly stained alpha lipoprotein band were judged from the more heavily loaded paper. In most cases albumin and the serum globulins were also quantitatively evaluated (Salt, 1956) and in a few selected cases the serum protein profiles were also obtained by detailed (40-part) elution of the bromophenol blue-stained strips.

RESULTS

Normal Findings

Sera from 27 children in whom there was no reason to suspect an abnormality of lipid metabolism were examined. These specimens were not specifically collected for our studies and this accounts for the wide differences in the omega fraction, some specimens having been obtained in the fasting state, others after a meal containing fat.

Although the normal ranges (Table 1) for total

TABLE 1
NORMAL SERUM LIPOPROTEIN VALUES

Age Group	No. of Cases	Mean Concentration and Range (mg. per 100 ml. serum)					Ratio Beta/Alpha
		Total Lipid	Electrophoretically Separated Fractions				
			Omega	Beta	Alpha	Beta + Alpha	
1-6 months	6	492	41	285	166	451	1.7
		294-711	6-119	162-427	117-199	282-626	1.3-2.2
½-11 years	21	676	43	434	200	633	2.2
		466-860	0-105	302-576	152-283	466-815	1.6-3.0

FIG. 1.—Serum lipoprotein electrophoretic patterns (upper strip of each pair, stained with oil red O, and profile superimposed) and serum protein electrophoretic patterns (lower strip of each pair, stained with bromophenol blue). A. Normal (aged 5 years) showing the three lipid fractions. B. Nephrosis. C. Diabetic coma. D. Hepatic cirrhosis. E. Essential hyperlipaemia. F. Essential hypercholesterolaemia.

lipids and for the three separate fractions are wide, the ratio of the beta fraction to the alpha fraction is relatively constant. Fig. 1A shows the normal electrophoretic patterns after staining for proteins and lipoproteins, and the lipid profile obtained by the elution technique.

The positions of the normal lipoprotein bands in relation to the protein bands are characteristic. The stained beta lipoprotein band, which coincides in its position with the beta globulin of the protein-stained strip, has a compact appearance with a sharp leading edge. These characteristics distin-

TABLE 2
SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH NEPHROSIS

Patient	Serum Albumin (g./100 ml.)	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak and character of Beta LP Band*
		Total	Omega	Beta	Alpha		
C.M. (1) ..	0.4	2,036	102	1,893	41	47	A+
R.F. ..	0.8	1,722	86	1,464	172	8.5	A+
R.N. ..	1.0	1,348	27	1,159	162	7.2	A+
S.P. ..	1.3	3,430	103	3,259	68	48	A+
A.H. ..	1.3	1,691	237	1,387	67	20.5	A
G.M. ..	1.5	1,610	193	1,240	177	7.0	A+
V.H. ..	2.2	1,323	79	1,005	239	4.2	N+
C.M. (2) ..	4.0	1,372	137	947	288	3.3	N
J.L. ..	4.9	907	45	671	191	3.5	N
R.G. ..	5.2	933	9	681	243	2.8	N

* A indicates advanced position of the peak; N the normal position.
+ denotes leading edge advanced towards the anode.

guish the normal from many pathological patterns. During the first six months of life, the beta lipoprotein band usually appears less intense and tends to be more widely diffused, though in general the pattern resembles that described for older children.

The stained alpha lipoprotein band is more diffuse than the beta band and corresponds in position with that occupied by α_1 globulin and the adjacent half of the albumin. In our assessment of the various patterns, normal and pathological, quantitative changes in alpha lipoprotein have been of greater value than qualitative variations in the appearance of the alpha band, though in two instances, described later, the alpha lipoprotein exhibited a striking qualitative abnormality.

Nephrosis

Sera were examined from nine children, aged 2-13 years, at various stages of the disease or in remission. The results are presented in Table 2, arranged according to the concentration of serum albumin. Typically the total lipids were grossly increased owing to a great increase in the beta fraction. The alpha fraction was sometimes decreased and the beta/alpha ratio exceeded the normal.

Fig. 1B shows a typical pattern at the height of

the disease. In these cases the beta lipoprotein was seen at an advanced position, sometimes between α_2 globulin and beta globulin, sometimes even as far as α_2 globulin, although not infrequently these globulin fractions were imperfectly separated or even merged in a way characteristic of nephrosis. In some instances there was in addition to the beta peak a less distinct fraction extending toward the anode. (Patterns that showed this abnormality are indicated in the tables by (+) and the normal or advanced position of the beta peak by N or A respectively.) As the serum albumin concentration increased, the beta lipoprotein peak returned to its normal position and magnitude, and the alpha lipoprotein fraction increased to a more normal proportion.

Cystine Storage (Lignac-Fanconi) Disease

Four cases were examined; the results are arranged in Table 3 according to the blood urea levels. In Case D.R. the blood urea and the lipoprotein pattern were normal. In the other cases the beta lipoprotein fraction either migrated to an advanced position, or the beta peak appeared at the normal position and a less intensely stained part of the beta fraction was also present extending forward to the α_2 globulin position. In two

TABLE 3
SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH CYSTINE STORAGE (LIGNAC-FANCONI) DISEASE

Patient	Blood Urea (mg./100 ml.)	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak and Character of Beta LP Band*
		Total	Omega	Beta	Alpha		
P.R. (1) ..	140	1,152	58	876	218	4.0	N+
P.R. (2) ..	109	980	167	715	98	7.3	A
K.C. ..	90	1,225	110	919	196	4.7	A+
L.S. ..	52	686	55	480	151	3.2	N+
D.R. ..	39	665	13	399	253	1.6	N

* See footnote, Table 2.

TABLE 4

SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH IDIOPATHIC INFANTILE HYPERCALCAEMIA

Patient and Age in Months	Type of Case	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak and Character of Beta LP Band*
		Total	Omega	Beta	Alpha		
R.F. 8	Benign	882	62	688	132	5.2	A
L.S. (1) 3	Benign	515	77	402	36	11	A
L.S. (2) 4	Benign	711	199	398	114	3.5	A
M.S. 18	Severe	909	73	554	282	2.0	A
J.S. 6	Severe	1,568	204	1,286	78	16	A+

* See footnote, Table 2.

of the cases (P.R. and K.C.) the total lipid and the beta lipoprotein concentrations were high and the beta/alpha ratio was slightly or moderately raised.

Idiopathic Infantile Hypercalcaemia

Four cases were examined, two (R.F. and L.S.) of the type described by Lightwood (1952) and two of the type described by Fanconi and Girardet (1952) and Schlesinger, Butler and Black (1952). Our results are given in Table 4. In all the cases the beta lipoprotein peak migrated to an advanced position and in three instances the serum total lipids were elevated.

Lipochondrodystrophy (Hurler's Syndrome)

Three patients were examined (Table 5); Case A.L. was studied on four occasions between the ages of 4 and 15 months. The other two patients were brothers (P.W., J.W.) and serum from their unaffected mother (Mrs. D.W.) was also examined. Results from the mother were normal; those of her children, as well as those of Case A.L., all showed a moderately increased beta/alpha ratio, a decreased alpha fraction and a normal or slightly increased beta fraction. This finding is of interest as, to our knowledge, a serum abnormality has not previously been described in this condition.

Mobilization (Transport) Lipaemia

Hypoproteinaemic Oedema (Due to Malnutrition). This boy (Case M.H., Table 6) was admitted at the age of 13 months with generalized oedema due to hypoproteinaemia (serum proteins: total 3.7, albumin 1.9, globulin 1.8 g. per 100 ml.). Since the age of 9 months he had refused most food and steadily lost weight. The first examination of serum lipids was made shortly after admission. The total lipids and beta fraction were high. In the electrophoretic protein strip a fraction was observed which had migrated in advance of the albumin, and in the corresponding position on the strip stained by oil red O a lipid fraction was present. This fraction appeared to be a modified alpha lipoprotein probably possessing an increased mobility as a result of acquisition of extra fatty acid components derived from lipolysis of fat mobilized from the depots. With treatment the boy's condition improved, the serum albumin increased and the oedema cleared. A month later the lipoprotein electrophoresis had returned to normal.

Chylothorax. In this 5-year-old boy (Case C.H.) the chylothorax was the result of a lymphangiomatous malformation of the thoracic duct. For eight weeks enormous volumes of chyle had accumulated in, and were aspirated from, the pleural cavity.

TABLE 5

SERUM LIPID AND LIPOPROTEIN FRACTIONS IN LIPOCHONDRODYSTROPHY (HURLER'S SYNDROME)

Patient	Age	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak, and Character of Beta LP Band*
		Total	Omega	Beta	Alpha		
A.L.	4 months	882	238	503	141	3.6	N+
"	7 "	760	15	578	167	3.5	N
"	9 "	809	113	575	121	4.7	N
"	15 "	662	40	543	79	6.8	N
P.W.	20 "	662	113	437	112	3.9	N
J.W.	10 years	662	46	483	133	3.7	N
Mrs. D.W. ..	Mother of P.W., J.W.	760	68	494	198	2.5	N

* See footnote, Table 2.

TABLE 6

SERUM LIPID AND LIPOPROTEIN FRACTIONS IN CONDITIONS WITH MOBILIZATION (TRANSPORT) LIPAEMIA

Patient	Condition	Characteristic Serum Chemistry	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Lipoprotein Peaks and Character of Bands*	
			Total	Omega	Beta	Alpha		Beta	Alpha
M.H. (1)	Malnutrition, oedema, hypo-proteinaemia	Albumin 1.9%	1,112	6	912	194	4.7	N+	Pre-albumin
(2)	Improved	Albumin 4.2%	671	67	429	175	2.5	N	N
C.H. (1)	Chylothorax	Albumin 2.1%	1,103	121	805	177	4.6	N+	N
(2)	After successful treatment	Albumin 2.4%	613	25	527	61	8.6	A	N
A.S. (1)	Idiopathic hypoproteinaemia (?hypercatabolic), oedema	Albumin 1.8%	1,250	325	800	125	6.4	A+	N
(2)	Improved	Albumin 3.6%	711	213	320	178	1.8	N	N
M.G. (1)	Diabetes mellitus, in coma ..	Sugar 1,570 mg. %	1,005	151	653	201	3.3	A+	Pre-albumin
(2)	Out of coma	—	613	31	484	98	4.9	A+	N

*See footnote, Table 2.

‘Pre-albumin’ denotes advanced position of alpha lipoprotein, such that the alpha fraction extends beyond the leading edge of the albumin.

Table 6 shows the findings at this stage of the illness when he had lost approximately 10 lb. in weight, when his general condition was rapidly deteriorating and the serum albumin had fallen to 2.1 g. per 100 ml. The total lipids and the omega and beta fractions were increased. Presumably mobilization of depot fat was the cause of this perplexing finding of pronounced chylomicronaemia in the virtual absence of alimentary chylomicron absorption. Ultimately the condition cleared with deep x-ray therapy. Examination of the serum was repeated within a fortnight of the leak of chyle into the pleural cavity having been sealed. The total lipid and the beta and omega fractions had by then returned to normal levels, and the alpha fraction had decreased. It may be speculated that with recovery the mobilization of depot fat had ceased and therefore excessive lipaemia was no longer a feature. The low alpha fraction appearing during convalescence was regarded, in the light of other experience (see below), merely as a non-specific abnormality.

Idiopathic Hypoproteinaemia. This 3-year-old girl (Case A.S.) had developed generalized oedema five days before admission. Investigations suggested that the oedema was due to hypoproteinaemia of hypercatabolic origin (Ulstrom, Smith and Heimlich, 1956; Schwartz and Thomsen, 1957). Table 6 gives the findings shortly after admission when the total lipids and the omega and beta fractions were increased. The beta fraction was in the advanced position, the lipid pattern resembling that seen in

nephrosis. On treatment with a protein-supplemented diet she improved rapidly and was discharged after seven weeks, when the serum proteins and lipoproteins were normal except for persistence of a high omega fraction. We regarded this persistent chylomicronaemia as another instance of mobilization lipaemia.

Diabetic Coma. In Case M.G. (see Table 6 and Fig. 1c) the serum total lipids, the omega and beta fractions were high; and the beta lipoprotein band occupied an advanced position. In addition, a fraction of alpha lipid and protein was discernible in the pre-albumin position.

A few days later, when the patient was out of coma but the diabetes still not well controlled, the lipoprotein pattern was more nearly normal, though the beta fraction remained in the advanced position and the alpha fraction, now in the normal position, was low.

Glycogen Storage Disease. We were able to study a family of five siblings of whom two (J.M., S.M.) were affected by the disease with hepatic involvement. The results are given in Table 7, and for comparison the findings in the three normal siblings (G.M., A.M., Ja.M.) are included. In the affected children the total lipid and the beta fraction were increased and the alpha fraction decreased, all in contrast to the normal findings in the unaffected children.

A third and younger case (T.L.), in whom the diagnosis though not proven by liver biopsy was

TABLE 7

SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH GLYCOGEN STORAGE DISEASE AND IN UNAFFECTED SIBLINGS

Subject	Age in Years	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak, and Character of Beta LP Band*
		Total	Omega	Beta	Alpha		
Affected siblings:							
J.M. 	5	931	47	745	139	5.3	A +
S.M. 	3	1,029	82	854	93	9.2	N +
Unaffected siblings:							
G.M. 	6	784	55	510	219	2.3	N
A.M. 	4	613	37	386	190	2.0	N
Ja.M. 	2	441	44	260	137	1.9	N
Another probable case							
T.L. (1) 	1½	613	43	423	147	2.9	N
T.L. (2) 	2	760	129	350	281	1.2	N

* See footnote, Table 2.

very likely (hypoglycaemia, hepatomegaly, positive adrenaline test), has so far shown normal lipoprotein patterns at the ages of 18 months and 2 years (see Addendum).

Liver Disease

Congenital Obliteration of Bile Ducts. Two patients were examined (Table 8) and in both the beta fraction was increased and the alpha fraction decreased. In one case (J.M.) the total lipids were also increased; in the other (J.O.) the total lipids, when examined one week after a successful anastomotic operation, were normal.

Neonatal Hepatitis. Three cases were studied (see Table 8); patients A.B. and C.B. were twins.

A.B. was only mildly affected and apart from a slightly increased beta/alpha ratio showed no abnormality. His brother was severely affected and ultimately died with cirrhosis at the age of 4 months. During the acute hepatitis stage (C.B.I.) the total lipid and beta fraction were normal but the alpha fraction much reduced. In view of his unsatisfactory progress the effect of cortisone was tried in the hope that it might prevent the onset of cirrhosis; fading of the jaundice and improvement in the patient's general condition ensued. At this stage (C.B.2) the alpha fraction was greater and the beta/alpha ratio less abnormal. Within two weeks of discontinuing cortisone the jaundice deepened and ascites developed. Shortly before death (C.B.3)

TABLE 8.

SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH LIVER DISEASE

Patient	Age	Diagnosis		Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak, and Character of Beta LP Band*
				Total	Omega	Beta	Alpha		
J.M.	10 weeks	Congenital obliteration of bile ducts	Before operation	1,054	74	885	95	9.3	N
J.O.	3 months	"	1 week after successful operation	662	0	622	40	16.0	N
A.B.	7 weeks	Neonatal hepatitis	Mild	515	39	366	119	3.1	N
C.B. (1)	7 weeks	"	Severe	613	48	546	19	30.0	N
(2)	3 months	"	Improving	588	12	517	59	8.8	N
(3)	4 months	"	In relapse, shortly before death from cirrhosis	294	0	262	32	8.0	N
S.P.	10 months	"	Shortly before death from biliary cirrhosis	319	0	246	73	3.4	N
C.J.	9 years	Infective hepatitis		625	13	569	43	13.0	N
J.P.	7 years	"		637	0	561	76	7.3	N
S.S.	10 years	Cirrhosis, " probably post-hepatitis	Shortly before death	83	29	52	2	35.3	N
M.H.	8 years	Cirrhosis, post-hepatitis		368	88	180	100	1.8	N
M.T.	11 years	Congenital hypoplastic anaemia; transfusion haemosiderosis; some evidence of liver damage		1,715	189	1,406	120	12.0	A +

* See footnote, Table 2.

TABLE 9
SERUM LIPID AND LIPOPROTEIN FRACTIONS IN PATIENTS WITH VARIOUS OTHER CONDITIONS

Patient	Diagnosis	Serum Lipid and Lipoprotein (mg. per 100 ml.)				Ratio Beta/Alpha	Position of Beta LP Peak, and Character of Beta LP Band*
		Total	Omega	Beta	Alpha		
D.A.	Essential hyperlipaemia	2,149	2,118	21 ?	10 ?	2.0 ?	Indeterminate
R.H.	Essential hypercholesterolaemia	1,323	53	1,045	225	4.6	N++
J.H.	Essential hypercholesterolaemia	1,980	20	1,584	376	4.1	N++
D.I.	Cretinism	980	69	813	98	8.3	N++
J.B.	Thyrotoxicosis	152	5	117	30	3.9	N++
P.F.	Reticulum-cell sarcoma	686	82	501	103	4.5	N++
P.H.	Infantile cortical hyperostosis	539	11	458	70	6.5	N++
C.M.	Dermatomyositis	515	36	376	104	3.7	N++
P.S.	Chronic respiratory infection	588	29	494	65	7.6	N

* See footnote, Table 2.

the total lipid and all fractions decreased markedly.

Another case (S.P.) was also examined shortly before her death from cirrhosis following neonatal hepatitis; there was a similar marked decrease in all lipid fractions.

Infective Hepatitis. Both cases (C.J., J.P., Table 8) were studied during the icteric stage and showed only a much diminished alpha fraction.

Post-Hepatitis Cirrhosis. Cases S.S. and M.H. (Table 8) had exceptionally low lipids due to diminution of the beta and alpha fractions. In both, the omega fraction had an unusual plateau-like shape (Fig. 1d). This pattern was not found in the two cases of cirrhosis following neonatal hepatitis, nor was it seen in any other condition studied.

Congenital Hypoplastic Anaemia (? Liver Damage). M.T. (Table 8), an 11-year-old boy, had received blood transfusions at approximately six-weekly intervals since 3 months of age; haemosiderosis had developed and possibly liver damage. An increased serum globulin concentration (2.1 g. per 100 ml.) strongly suggested such damage, though a normal serum gamma albumin level (4.8 g. per 100 ml.) indicated that advanced cirrhosis was not present.

The total lipid and the beta and omega fractions were grossly increased and the beta band appeared in an advanced position. An interpretation of these findings is difficult, as the pattern does not conform to those found in our other cases of liver disease. The changes seem unlikely to be a reflection of the primary abnormality of erythrocyte formation, as the lipoprotein patterns in two other patients, who were younger and had received fewer transfusions, were normal. Further studies are needed to elucidate the nature of this unexpected abnormality.

Essential Hyperlipaemia

The pattern (Fig. 1e) (in Case D.A., Table 9), which is diagnostic, is characterized by an enormously increased omega fraction, with virtual absence of beta and alpha lipoproteins. The values for the beta and alpha fractions were extremely low and may be inaccurate owing to difficulties in the separation of these fractions in the presence of an overwhelming quantity of chylomicron material.

Essential Hypercholesterolaemia

In these patients (Cases R.H. and J.H., Table 9) the beta fraction was greatly increased. At first sight the pattern (Fig. 1f) appeared similar to that seen in nephrosis but in contrast to the nephrotic pattern the beta lipoprotein was normal in position relative to the globulins. Moreover the alpha fraction was not diminished, and in Case J.H. even elevated.

Cretinism

In Case D.I., aged 15 months, untreated (Table 9), the beta lipoprotein was increased but occupied its normal position. The alpha fraction was diminished, so that the beta/alpha ratio showed a definite elevation. These findings were of special interest, as the serum cholesterol (186 mg. per 100 ml.) was within normal limits.

Thyrotoxicosis

In Case J.B., aged 11 years (Table 9), in contrast to the findings in hypothyroidism, the total lipids were remarkably low and the depletion was noticeable in all the fractions. Serum cholesterol in this patient was 127 mg. per 100 ml., a value likewise within normal limits.

Miscellaneous Conditions

The commonest abnormality found in various conditions comprised a moderate decrease in alpha

lipoprotein, less marked than in nephrosis or jaundice, coexistent with a beta fraction normal in quantity and position. In consequence there was a slight or moderate increase of the beta/alpha ratio.

Cases P.F., P.H., C.M., P.S. (see Table 9) illustrate the diversity of conditions in which this abnormality was found.

DISCUSSION

In some conditions, this technique of lipoprotein electrophoresis yielded diagnostic information; in others, serial patterns, though not diagnostic, were helpful in assessing progress. In our group of cases, the technique was of diagnostic value in nephrosis, idiopathic infantile hypercalcaemia, glycogen storage disease, essential hyperlipaemia, essential hypercholesterolaemia, cretinism, thyrotoxicosis, some forms of liver disease and possibly in Hurler's syndrome.

In the difficult differential diagnosis of congenital obliteration of the bile ducts from neonatal hepatitis this technique might prove helpful. The results suggested that in the early months of life, when correct diagnosis is so important, serum total lipid and beta lipoprotein tend to be higher in obliteration than in hepatitis. In the later stages, as cirrhotic changes complicate both hepatitis and unrelieved obstruction, the total lipid and the beta fraction are likely to become decreased in both conditions and lipoprotein electrophoresis is then unlikely to give diagnostic help.

An unusual plateau shape in the omega fraction profile was observed in association with low total lipids in two older cases of post-hepatitis cirrhosis. This feature was not seen in the infants who developed cirrhosis following obliteration or neonatal hepatitis.

Less specific abnormalities were found in many conditions, e.g., cystine storage disease in the stage of severe glomerular damage, and in various forms of mobilization lipaemia. In such cases repeated examinations were helpful in assessing progress.

In various conditions, notably nephrosis, cystine storage disease, infantile hypercalcaemia, diabetes mellitus and malnutrition with transport lipaemia, the serum beta lipoprotein was found to possess an enhanced electrophoretic mobility. The alpha lipoprotein was seen likewise to vary somewhat in its mobility, and in two cases was advanced so markedly that a part appeared ahead of the serum albumin. Fast-moving beta and alpha fractions have been produced by intravenous injection of heparin (Herbst, Lever, Lyons and Hurley, 1955) and by intravenous laminarin sulphate M (Besterman and Evans, 1957). Presumably a lipolytic process was

thereby potentiated, the liberated fatty acids being attached to beta lipoprotein, and in extreme cases to alpha lipoprotein also, with consequent enhancement of their mobility. These findings suggest that in our cases, in response to a nutritional need, mobilization from the lipid stores was occurring with subsequent spontaneous lipolysis.

SUMMARY

A method for separating serum lipoproteins by electrophoresis is described including quantitative evaluation of the fractions and the total lipid.

The normal findings in infants and children are presented, and abnormalities found in various disease states are described. The procedure was of diagnostic value in nephrosis, idiopathic infantile hypercalcaemia, glycogen storage disease, essential hyperlipaemia and hypercholesterolaemia, thyroid disorders and some forms of liver disease. In these and certain other conditions, serial examinations were of value in assessing progress.

In some forms of renal disease and mobilization lipaemia the beta lipoproteins, and sometimes also the alpha lipoproteins, were found in a position ahead of the normal. The significance of this finding is discussed.

We are grateful to Professor J. M. Smellie for his support and interest throughout this work; and thank Dr. H. W. Everley Jones, Dr. J. M. Garvie and members of the consultant staff of the Children's Hospital for access to their patients.

Addendum

Since submitting this paper for publication we have re-investigated Case T.L. (Table 7), the boy in whom a diagnosis of glycogen storage disease had seemed likely, despite the normal lipoprotein electrophoresis. There is now no hepatomegaly, the adrenalin test is negative, and the diagnosis is ruled out. The high diagnostic value of lipoprotein electrophoresis in glycogen storage disease is thus emphasized.

REFERENCES

- Adlersberg, D., Bossak, E. T., Sher, I. H. and Sobotka, H. (1955). *Clin. Chem.*, **1**, 18.
- Besterman, E. M. M. and Evans, J. (1957). *Brit. med. J.*, **1**, 310.
- Dangerfield, W. G. and Smith, E. B. (1955). *J. clin. Path.*, **8**, 132.
- Fanconi, G. and Girardet, P. (1952). *Helv. paediat. Acta*, **7**, 314.
- Gottfried, S. P., Pope, R. H., Friedman, N. H., Akerson, I. B. and Di Mauro, S. (1955). *Clin. Chem.*, **1**, 253.
- Grassmann, W., Hannig, K. and Knedel, M. (1951). *Dtsch. med. Wschr.*, **76**, 333.
- Herbst, F. S. M., Lever, W. F. and Hurley, N. A. (1955). *J. invest. Derm.*, **24**, 507.
- , Lyons, M. E. and Hurley, N. A. (1955). *J. clin. Invest.*, **34**, 581.
- Kunkel, H. G. and Slater, R. J. (1952). *Ibid.*, **31**, 677.
- and Tiselius, A. (1951). *J. gen. Physiol.*, **35**, 89.
- Laurell, C. B. (1955). *Scand. J. clin. Lab. Invest.*, **7**, 257.
- Lightwood, R. (1952). *Archives of Disease in Childhood*, **27**, 302.
- Rafstedt, S. (1955). *Acta. paediat. (Stockh.)*, **44**, Suppl. 102.
- Salt, H. B. (1956). *Clin. Chem.*, **2**, 35.
- Schlesinger, B., Butler, N. and Black, J. (1952). *Helv. paediat. Acta*, **7**, 335.
- Schwartz, M. and Thomsen, B. (1957). *Brit. med. J.*, **1**, 14.
- Studnitz, W. von (1955). *Scand. J. clin. Lab. Invest.*, **7**, 329.
- Swahn, B. (1952a). *Ibid.*, **4**, 98.
- (1952b). *Ibid.*, **4**, 247.
- Ulstrom, R. A., Smith, N. J. and Heimlich, E. M. (1956). *A.M.A. J. Dis. Child.*, **92**, 219.