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PLASMA LIPID AND LIPOPROTEIN CHANGES  
IN OBSTRUCTIVE JAUNDICE:  
IMMUNOCHEMICAL IDENTIFICATION OF ABNORMAL  
ALPHA LIPOPROTEIN FORMS

by

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VOLUME I

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## PREFACE

The work described in this thesis was performed during my tenure of a British Heart Foundation and American Heart Association British-American Exchange Fellowship, September 1967 - August 1968.

The Fellowship was based in the Section on Lipoproteins, Laboratory of Molecular Disease, National Heart Institute, Bethesda, Maryland, U.S.A. (Director - Dr. Donald S. Fredrickson).

The general aspects of the project were outlined to me, on my arrival, by Dr. Robert I. Levy, Head of the Section on Lipoproteins. The subsequent planning of the investigation and its daily pursuit were my responsibility. Periodically its evolution was discussed with Dr. Levy. Except where specifically acknowledged, all the investigations described in this thesis were carried out personally.



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE  
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To whom it may concern:

Dr. Basil Rifkind has been a Visiting Scientist in the Molecular Disease Branch of the National Heart Institute for the past year. During this time he has participated in some of our patient care activities.

He has directed a majority of his time toward the investigation of the lipoprotein abnormalities that occur in obstructive liver disease. With guidance, essentially all of Dr. Rifkind's work, both immunochemical and chemical, has been done by him, for the most part without technical assistance.

Dr. Rifkind has succeeded in assembling some new information regarding the nature of lipoprotein complexes and lipoprotein abnormalities that occur in obstructive liver disease. These observations, I am sure, will contribute substantially toward our understanding of the seemingly paradoxical plasma lipid abnormalities previously reported in liver disease.

Please feel free to contact us if you have any questions.

Sincerely yours,

A handwritten signature in cursive script, reading "Robert I. Levy".

Robert I. Levy, M.D.  
Head, Section on Lipoproteins  
Deputy Clinical Director  
National Heart Institute

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Subject nos. 6, 11 and 15 attended the Clinical  
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## SUMMARY

1. Alpha lipoprotein levels have been variously described as increased or decreased in the plasma of subjects with jaundice due to intra- or extra-hepatic biliary obstruction, including biliary cirrhosis, the findings varying with the method used.

2. To resolve this paradox, a chemical and immunochemical study of the changes that occurred in the plasma lipids and lipoproteins of 21 subjects with various types of obstructive jaundice was performed.

Three patterns of lipoprotein disturbances were recognised.

3. In 9 subjects an abnormal alpha lipoprotein form was detected by immunoelectrophoretic and immunodiffusion studies within the  $d$  1.006 - 1.063 fraction, together with beta lipoprotein. This low density alpha lipoprotein displayed cathodal or retarded electrophoretic mobility in agar and agarose gels respectively, and abnormal features in immunodiffusion studies. These subjects also showed, on paper electrophoresis/

electrophoresis of their d 1.006 - 1.063 fraction, an additional lipoprotein band migrating between the origin and the beta lipoprotein band, or cathodally; this band displayed unusual staining characteristics in that it usually stained for protein but not for lipid although the latter was shown to be present. It is thought to correspond to the abnormal alpha lipoprotein detected on immunoelectrophoresis. These abnormalities disappeared with relief of jaundice.

4. An abnormal alpha lipoprotein form was also detected at d 1.006 - 1.063 in the plasma of 2 subjects; unlike the previous form it showed normal electrophoretic mobility in agarose and agar gels, and in paper in which it stained for lipid and for protein. Its immunodiffusion characteristics were unaltered.

5. In 10 subjects alpha lipoprotein was not identified outwith its normal density class, and in this group/

group elevated levels of beta lipoprotein of altered composition were found establishing the occurrence of a pure hyperbetalipoproteinaemia.

6. Quantitative studies of the plasma lipid and lipoprotein levels, and of lipoprotein composition showed several significant differences between the groups, with the maximum disturbances occurring in the subjects with the low density alpha lipoprotein of altered electrophoretic mobility.

7. On the basis of these findings it is suggested that the appearance of alpha lipoprotein forms of abnormally low density, with or without altered electrophoretic mobility, results from the overlipidation of alpha lipoprotein secondary to biliary obstruction.

8. The demonstration of an alpha lipoprotein form of low density, altered electrophoretic mobility and unusual staining properties resolves the previous conflicting reports regarding alpha lipoprotein changes/

changes in obstructive jaundice.

9. A lipoprotein of beta mobility was found within the  $d < 1.006$  fraction of 11 subjects, similar to the "floating" beta lipoprotein of familial Type III hyperlipoproteinaemia.

10. Subjects with jaundice due to extra- or intra-hepatic biliary obstruction could not be distinguished from those with biliary cirrhosis by their lipid and lipoprotein abnormalities.

## NOMENCLATURE

The plasma lipoproteins are usually classified in operational terms (Table 1.1 and see Chapter 1) according to their electrophoretic mobility on paper (Lees and Hatch, 1963), or their density characteristics as exploited by use of the preparative ultracentrifuge, or their flotation properties as measured in the analytical ultracentrifuge.

The 4 major lipoprotein classes defined by any of these systems broadly correspond to those defined by the other systems (Hatch et al, 1967).

Although such classifications have considerable validity, are usually interchangeable, are generally accepted and have been of great use, they are limited in their failure to provide an entirely adequate division which reflects the metabolic interrelationships between the various classes (Schumaker and Adams, 1969). Another major deficiency of operational classifications has been/

been recognised with the recent demonstrations that lipoproteins with altered physico-chemical properties occur in certain diseases. For example, in familial Type III hyperlipoproteinaemia ("broad beta" disease), a lipoprotein with the electrophoretic mobility of beta lipoprotein is found at  $d < 1.006$  (Fredrickson et al, 1967). Similarly a small amount of beta lipoprotein has been found to float at  $d 1.006$  in subjects with Tangier Disease or Hereditary High Density Lipoprotein Deficiency (Fredrickson et al, 1967). Likewise, in abetalipoproteinaemia, alpha lipoprotein has been identified, on the basis of its immunochemical specificity and its amino-acid composition, within the density range  $1.019 - 1.063$ , normally solely occupied by beta lipoprotein (Levy et al, 1966).

With this in mind the following nomenclature has been adopted for the present study. The abbreviations VLDL, LDL and HDL refer only to any lipoproteins/

lipoproteins observed within the density classes  $< 1.006$ ,  $1.006 - 1.063$  and  $> 1.063$  respectively; they do not define particular lipoprotein classes. The terms chylomicrons, beta, pre-beta and alpha lipoproteins define the 4 major classes of lipoproteins with properties conforming to those set out in Table 1.1 except where evidence is presented for alteration in one or more of these properties.

An exception to this nomenclature is when the findings of other workers are described, when the terminology employed by them has been retained. This has been necessary since in some reports the lipoproteins are defined purely on the basis of the method used to study them and it has occasionally proved difficult to translate the lipoprotein class as defined by a given worker into the nomenclature adopted in the present paper.

SECTION I

INTRODUCTORY REVIEW

CHAPTER I

THE PLASMA LIPOPROTEINS:

THEIR PROPERTIES AND FUNCTIONS

This chapter classifies the plasma lipoproteins, gives a detailed account of each of them and presents current views of their major functions. It is not meant to provide a comprehensive description of all aspects of the plasma lipoproteins, but attempts to set them in perspective, emphasising those features which are or may be of relevance in understanding the changes which occur in obstructive jaundice and biliary cirrhosis.

Plasma lipids      The major plasma lipids are free cholesterol, cholesterol ester, triglyceride and phospholipid. The functions of these various plasma lipids still remain to be fully comprehended (Nichols, 1969). Free cholesterol is found in almost all tissues. It is a precursor of the steroid hormones and of the bile acids. Its presence in many cell membranes suggests a structural role there. Cholesterol ester is mainly found in the plasma, liver and adrenal glands. Its function is obscure (Goodman, 1965). In man, in harmony with many species, the proportion of plasma cholesterol maintained in ester form is remarkably constant (Goodman, 1965).

Triglyceride participates in energy metabolism; on hydrolysis it yields free fatty acids which are utilised by various tissues including skeletal and cardiac muscle as a major source of energy. Fatty acid released from triglyceride and not oxidised may be re-esterified in liver or in adipose tissue ultimately providing a reservoir of free fatty acid in the form of stored glyceride.

The/

The phospholipids are found in many cellular and subcellular membranes where they appear to have a structural role. Their amphipathic properties with their tendency to orientate at lipid-water interfaces are strongly suggestive of a stabilising influence on lipoprotein complexes.

Transport tasks of lipoproteins      Considerable amounts of lipid require to be transported through the plasma. Fredrickson (1969) has estimated the net daily transport of the various lipids in the adult; 70 - 150 G of exogenously derived triglyceride pass through the plasma each day in a series of fluxes following the ingestion of triglyceride-containing meals. The turnover of endogenous triglyceride, mainly of hepatic origin is about 25 - 50 G/day. The daily turnover of free and ester cholesterol is approximately 1 G.

The solubilisation of highly apolar lipids such as triglyceride, cholesterol and cholesterol ester in aqueous plasma is achieved by the formation of macromolecular complexes called lipoproteins.

Plasma lipoproteins      Four major lipoprotein classes are/

are generally recognised in the normal subject. They are usually defined in operational terms (Table 1.1). According to their mobility during electrophoresis on paper they are described as beta lipoproteins, pre-beta lipoproteins and alpha lipoproteins; the 4th class, the chylomicrons, fails to migrate on paper (Lees and Hatch, 1963). Alternatively, they may be separated according to their differing densities, using the preparative ultracentrifuge (Havel et al, 1955) into 4 classes, corresponding to those defined by electrophoresis and named low density lipoproteins (LDL), very low density lipoproteins (VLDL), high density lipoproteins (HDL) and chylomicrons. A 3rd system classifies them according to their behaviour in the analytical ultracentrifuge; they are assigned  $S_f$  values (Lindgren et al, 1951, DeLalla and Gofman, 1954) which express their rate of flotation per unit centrifugally in a solution of sodium chloride (density 1.063) at temperature 26°.

In addition to these 4 generally recognised lipoprotein classes, there may exist a lipoprotein of  
d/

$d > 1.21$  ("very high density lipoprotein") (Fredrickson et al, 1967).

Plasma from a healthy fasting subject contains 2 major lipoproteins, the beta and alpha lipoproteins.

Beta lipoprotein (LDL,  $S_f$  0 - 20 lipoprotein)

The beta lipoproteins migrate with the mobility of beta globulin in various electrophoretic media including paper (Lees and Hatch, 1963). They occupy the density range 1.006 to 1.063 (DeLalla and Gofman, 1954). Their lipid and protein composition, expressed as a percentage of the dry weight of the total lipoprotein is shown in Table 1.2. Almost half of the complex consists of cholesterol of which about 80% is esterified (Goodman, 1965). The balance consists of about equal amounts of protein and phospholipid (approximately 22% of each) and a small amount of triglyceride. These figures do not include the carbohydrate moiety which is also present in beta lipoprotein (Marshall and Kummerow, 1962) and whose significance is undetermined.

The protein moiety of beta lipoprotein (apoprotein B) is thought to consist of several identical or similar polypeptides characterised by carboxyterminal serine and aminoterminal/

aminoterminal glutamic acid and by a total amino acid composition which is quite different from that of the HDL apoprotein (Fredrickson et al, 1967, Oncley and Harvie, 1969).

Structural studies have been hampered by the difficulty in obtaining a delipidated apoprotein which retains its aqueous solubility, the stripping of lipid from the complex resulting in a gel-like material insoluble in water or urea (Gotto, 1969). Recently various chemical modifications of the apoprotein such as succinylation and/or the use of detergents such as sodium dodecyl and decyl sulphate have yielded essentially lipid free apoproteins (see Gotto, 1969 for summary of these methods). The soluble apoprotein of low density lipoprotein (apoLDL) retains prominent immunological and optical characteristics of native LDL (Gotto, 1969). The conformation of LDL has been investigated using optical methods such as infra-red and circular dichroic spectroscopy. They have revealed a significant amount of pleated sheet anti-parallel chain beta-structure with some random/

random and, probably, alpha helical structure (Gotto et al, 1968). Delipidation results in an increase of random structure.

A primarily spherical form of mean diameter 216 Å with no specific sub-unit structure has been proposed for beta lipoprotein (Nichols, 1969) on the basis of electron microscopy. Different electron microscopic findings have been described by Pollard et al (1969) who on the basis of ultracentrifugal and electron microscopic studies of human LDL and several of its derivatives, have formulated a model of the lipoprotein in which 20 protein sub-units are arranged in a dodecahedron pattern with icosahedron symmetry. The lipoprotein surface is held to be occupied by both protein and lipids, especially phospholipid, with apolar lipids (cholesterol esters and triglyceride) in the interior of the molecule.

Beta lipoprotein is strongly antigenic, even trivial amounts stimulating the production of a potent antibeta lipoprotein antiserum (Fredrickson et al, 1967). Purified agar or agarose (sulphate-free/

free agar) provide the best media for immunochemical studies of beta lipoproteins; other media suffer from the disadvantage of interacting with the lipoprotein.

Antisera to the beta apoprotein either form lines of complete identity with beta lipoprotein and the beta apoprotein or detect very minor differences suggesting that the protein is the major immunochemical determinant (Gotto, 1969). Nevertheless, antisera to beta lipoprotein produce only lines of partial identity between beta lipoprotein and the beta apoprotein and it has been suggested that delipidation leads to the removal of haptenic reactants or to conformational changes in the peptide.

Beta lipoprotein probably originates in the liver and the intestine (Windmueller and Levy, 1967) but may solely or partially appear in the plasma as a product of pre-beta lipoprotein metabolism. A precursor-product relationship between pre-beta lipoprotein and beta lipoprotein has been shown by kinetic studies of low density lipoproteins, and during the acute in vivo hydrolysis/

hydrolysis of pre-beta lipoprotein induced by the intravenous administration of heparin, lipoprotein products are released including one with the ultra-centrifugal, paper electrophoretic and chemical composition of beta lipoproteins (Nichols et al, 1968).

Beta lipoprotein or its apoprotein is not uniquely confined to the low density fraction bearing its name; it is also identifiable, following delipidation, in pre-beta lipoproteins (Levy et al, 1966) and is probably a constituent of chylomicrons (Fredrickson et al, 1967).

#### Alpha lipoprotein (HDL)

Alpha lipoproteins have  $\alpha_1$  mobility on free electrophoresis and in media such as paper and starch. They are isolated within the density range 1.063 - 1.21. They have been sub-divided into HDL<sub>2</sub> (d 1.063 - 1.12) and HDL<sub>3</sub> (d 1.12 - 1.21) sub-classes (DeLalla and Gofman, 1954), but the latter may be artefactually derived from the former (Levy and Fredrickson, 1965). Unlike the other lipoproteins of lower density they are/

are not precipitated by polyanions such as heparin but remain in the supernate, a property which is exploited for their quantitation (Burstein and Samaille, 1960).

The lipid and protein composition of alpha lipoprotein is shown in Table 1.2. About 50% of the complex is protein; the balance consists of approximately 30% phospholipid and 18% cholesterol, about 80% of which is esterified, and a small amount of triglyceride. The protein moiety also contains over 3% carbohydrate by weight (Epstein and Block, 1959).

It was formerly considered that the protein moiety of alpha lipoprotein (apoprotein A) solely consisted of polypeptide sub-units containing amino-terminal aspartic acid and carboxyterminal threonine (apoHDL-Thr) (Shore, 1957). Recently an additional polypeptide with carboxyterminal glutamine (apoHDL-Gln) has been described (Shore and Shore, 1968). Approximately equal amounts of apoHDL-Thr and apoHDL-Gln account for over 90% of the protein of HDL<sub>3</sub>.

Optical/

Optical studies of native HDL show it to be rich in alpha-helical conformation (Gotto, 1969). Circular dichroic measurements of apoHDL-Gln and apoHDL-Thr show the former to contain considerably less alpha-helical structure (40-50%) than the latter ( $> 90\%$ ). These results inversely correlate with the content of certain non-helical aminoacids in the peptides (Gotto and Shore, 1969). It has been postulated that apoHDL-Gln may be particularly involved in the binding of HDL lipid (Gotto, 1969; Gotto and Shore, 1969).

Cross-reacting antisera can be prepared against alpha lipoprotein or its soluble apoprotein. Alpha lipoprotein is less potent than beta lipoprotein in stimulating the production of antisera, perhaps related to its lower lipid content. It is difficult to obtain a completely pure preparation of alpha lipoprotein since antisera raised against typical preparations often display additional anti-beta lipoprotein reactivity (Fredrickson et al, 1967). However, it is generally agreed that alpha lipoprotein is/

is antigenically distinct from beta lipoprotein (Fredrickson et al, 1967, Margolis, 1969). Fresh plasma normally contains a single immunological form of alpha lipoprotein. Following recent storage or various laboratory manipulations of plasma, an additional slower migrating form appears on immunoelectrophoresis of the plasma. Levy and Fredrickson (1965) have designated these two alpha lipoprotein forms as alpha LP<sub>A</sub> and alpha LP<sub>B</sub> respectively, suggesting that the latter represents a partially delipidated alpha lipoprotein containing a smaller polymer of the basic sub-unit of the apoprotein.

On electron microscopy, alpha lipoprotein displays aggregates consisting of 4 - 6 spherical sub-units (Nichols, 1969).

The biological half-life of alpha lipoprotein is about 4 days, similar to that of beta lipoprotein, considerably slower than that of chylomicrons or pre-beta lipoproteins but faster than that of almost all the other plasma proteins (Gitlin et al, 1958).

Alpha lipoprotein or its protein moiety is also not/

not specific to the high density lipoprotein class bearing its name. It is also identifiable in and may stabilise pre-beta lipoprotein, and may be a component of chylomicrons (Fredrickson et al, 1967).

A congenital and complete deficiency of normal alpha lipoprotein is found in the recessively inherited disorder, Tangier Disease; the striking storage of cholesterol ester which occurs in the reticulo-endothelial system of affected individuals suggests that alpha lipoprotein has a lipid transport function especially relating to cholesterol. Recently additional functions have been postulated for HDL or its apoprotein; it has been suggested that bound apoprotein A activates lipoprotein lipase and the lecithin:cholesterol acyl transferase enzyme (Schumaker and Adams, 1969).

#### Chylomicrons ( $S_f$ 400)

Chylomicrons (Gage, 1920) are defined as particulate fat of alimentary origin (Dole and Hamlin, 1962). Their electrophoretic mobility varies according to the medium used and according to whether they are/

are derived from plasma or lymph (Frazer, 1949, Kunkel and Slater, 1952). On electrophoresis in paper, employing albumen-containing buffer, the chylomicrons remain at the origin (Lees and Hatch, 1963).

Chylomicrons are of  $d < 0.95$  and are the lightest lipoprotein class. They are assigned an  $S_f$  value greater than 400. It is not possible to distinguish, using the above criteria, the smallest chylomicrons from the largest pre-beta lipoproteins. Their low density reflects their exceedingly high lipid and low protein content. The precise nature of their protein component has been difficult to establish since some or all of the proteins observed may merely have been absorbed on to the surface of the particles when they enter lymph and plasma, analogous to the similar proteins acquired by a coconut oil emulsion following its incubation in plasma (Scanu and Page, 1959). However, it is now generally held that both alpha and beta lipoproteins (or their apoproteins) are constituents of chylomicrons (Fredrickson et al, 1967). The postulated structural role of beta lipoprotein in chylomicrons/

chylomicrons is considerably strengthened by the observation that subjects with a congenital deficiency of beta lipoprotein (abetalipoproteinaemia) are unable to form chylomicrons (Farquhar and Ways, 1966).

Chylomicrons have a spherical shape on electron microscopy and a diameter ranging from 1,200 - 11,000 Å although most of the triglyceride is found in particles of 1,500 - 4,000 Å (Nichols, 1969). The particles are rapidly cleared from the plasma, displaying a biological half-life of 5 - 15 minutes. The major clearance pathway involves a hydrolytic step which does not take place in the plasma but which appears to occur on the endothelial surface of capillaries under the influence of lipoprotein lipase (Havel, 1965). This enzyme which can be made to appear in the plasma shortly after the injection of small amounts of heparin, splits the triglyceride into glycerol and fatty acids. Recent studies suggest that lipoprotein lipase activity represents the net activity of several enzymes, a monoglyceride lipase/

lipase having been described in addition to the classical triglyceride lipase (Greten et al, 1969).

Pre-beta lipoprotein (VLDL,  $S_f$  20 - 400)

Pre-beta lipoprotein migrates with  $\alpha_2$  globulin on electrophoresis in starch or paper, is found between  $d$  0.95 - 1.006, and has an  $S_f$  value of 20 - 400. As mentioned previously there is overlap between the largest pre-beta lipoprotein particles and the smallest chylomicrons with respect to some of these characteristics. When pre-beta lipoprotein concentration rises the particle size increases and material described as "trail" is found on paper electrophoresis migrating from the pre-beta zone towards the origin (Lees and Hatch, 1963).

Pre-beta lipoproteins are also rich in triglyceride which, in contrast to that of the chylomicrons, is mainly endogenously derived, the predominant source being hepatic synthesis from fatty acid or carbohydrate precursors. Triglyceride accounts for approximately 65% by weight of pre-beta/

pre-beta lipoprotein, the amount rising as the particle size increases. Cholesterol accounts for approximately 13%, protein 10% and phospholipid 12% (Table 1.2). The fatty acid composition of triglyceride differs from that of chylomicron triglyceride and is that of endogenously synthesised fat (Bierman et al, 1965).

The protein moiety of pre-beta lipoprotein is currently the object of considerable study. Formerly, beta lipoprotein (or its apoprotein) was regarded as the main protein component. Levy et al (1966) subsequently showed that, following careful delipidation of VLDL, alpha lipoproteins were also detectable by immunochemical means. The  $\alpha_2$  electrophoretic mobility of this class has been attributed to the addition of the faster moving alpha lipoprotein to the beta lipoprotein containing complex.

Recently it has been demonstrated that about 50% of the protein in VLDL does not consist of the alpha and beta lipoprotein apoproteins, and 2 additional polypeptides/

polypeptides characterised as carboxyterminal valine and carboxyterminal alanine VLDL apolipoproteins have been described (Brown et al, 1969). A 5th polypeptide, carboxyterminal glutamine VLDL apolipoprotein, has just been reported (Brown et al, 1970).

CHAPTER 2

DISORDERED PLASMA LIPIDS AND LIPOPROTEINS IN  
OBSTRUCTIVE JAUNDICE

An account of the changes which have been described in subjects with extra-hepatic biliary obstruction and biliary cirrhosis is given in this chapter. Certain of the findings are dealt with in expanded detail in Chapters 6, 7 and 10.

The first link between disordered lipid metabolism and hepatic disease seems to have been forged by Thomas Addison and William Gull (1851) who described "A Certain Affection of the Skin" described as "Vitiligoidea" which occurred in two forms "Plana" and "Tuberosa". The skin lesions are well illustrated and would now be regarded as tuberous and planar xanthomata and xanthelasma. They associated the lesions with "hepatic derangement" and the details given suggest that this took the form of obstructive jaundice.

Eleven years later Austin Flint Jr. (1862) published experimental studies which led him to conclude that "cholesterine" was normally separated from the blood by the liver, to be excreted with the bile into the alimentary canal. The retention of cholesterine in the blood, "cholesteremia", he suggested was due to extensive structural disease of the liver, but he noted that, in some cases of "simple jaundice" (in retrospect, probably obstructive in type) cholesteremia also occurred.

In/

In 1918 Feigl showed the increase in serum cholesterol to be due mainly to an increase in free cholesterol so that the ratio of free:ester cholesterol was increased.

A profile of the lipid disturbances in biliary obstruction and biliary cirrhosis was provided by Man et al (1945). They found total cholesterol and lipid phosphorus, especially the latter, to be raised in obstruction and to return to normal following its relief. High levels of these lipids were also observed in some cases of biliary cirrhosis and in the early obstructive phase of infective hepatitis. Neutral fat levels, determined by difference, were usually above normal, sometimes greatly so in obstructive jaundice. Free cholesterol levels were absolutely increased and also relative to cholesterol ester levels, which were usually diminished and which rose as obstruction was relieved.

These observations were confirmed and extended as reviewed by Ahrens et al (1950). In patients with/

with biliary cirrhosis they found preponderant elevations of phospholipid levels. They remarked on the characteristic clarity of the sera of such patients despite great increases in lipid levels, and attributed it to the high phospholipid levels and the surfactant properties of this lipid class. The xanthomata observed in many of their patients were clearly shown to be a consequence of the hyperlipidaemia rather than its cause, a view formerly propounded by Thannhauser (1940).

Kunkel and Ahrens (1949) presented evidence for the transport forms of these elevated lipids, suggesting that they were carried with the beta globulins. Using free electrophoresis they found marked increases in the beta globulin components in the serum of 8 patients with biliary cirrhosis. Beta globulin levels were directly proportional to the total serum lipid concentration over a wide range of lipid levels. Partial delipidation of the serum resulted in a marked reduction in the beta globulin/

globulin components; the amount of lipid removed by this procedure relative to protein was greater than normal, providing the first hint that lipoprotein composition was disturbed in biliary cirrhosis. Sterling and Ricketts (1949) obtained similar results.

Later Kunkel and Slater (1952) applied the technique of zonal electrophoresis on starch to the study of lipoproteins and found, in 5 cases of biliary cirrhosis with hyperlipidaemia, a very high peak composed mainly of phospholipid and which migrated with an electrophoretic mobility approximately similar to normal beta lipoprotein. They also described a great reduction or complete absence of the alpha lipoprotein peak in the subjects and in 2 others with extra-hepatic obstructive jaundice. They suggested that the low cholesterol-phospholipid ratio of the beta component might be due to a new lipoprotein migrating with the same mobility as normal beta lipoprotein, or more likely, to beta lipoprotein/

lipoprotein having an altered lipid composition. Preliminary experiments suggested a lipid-protein ratio in this abnormal peak close to but slightly higher than that of normal beta lipoprotein.

The analytical ultracentrifuge was used to further define the lipoprotein abnormalities by McGinley et al (1952). A unique pattern was found characterised by high increases of  $S_f$  6 and  $S_f$  8 classes with a varying increase in the  $S_f$  10 - 17 lipoproteins and a sharp cut off at  $S_f$  17. They alluded to, but did not provide evidence for, the possibility that the  $S_f$  6 and  $S_f$  8 classes had a different structure and chemical composition from normal.

Eder et al (1955), in a detailed study, provided firm evidence for disordered lipoprotein composition in biliary cirrhosis and obstructive jaundice. An interesting paradox was also revealed by their work in that, depending on the method used, alpha lipoproteins were found to be high or low. They/

They used the Cohn fractionation procedure, method no. 10 (Cohn et al, 1950) to obtain fraction IV, V and VI, which normally houses alpha lipoprotein, and fraction I and III which normally houses beta lipoprotein. They showed a number of changes in the lipoproteins of both fractions.

The increased total serum cholesterol of their subjects tended to accumulate in fraction IV, V and VI. This fraction had a normal cholesterol:phosphorus ratio but all the cholesterol was in the free form. Normally alpha lipoprotein has a higher proportion of its cholesterol in the ester form compared with beta lipoprotein. On paper and on starch this fraction migrated with the mobility of beta globulin, alpha lipoprotein being negligibly small, though it rose with relief of the disease. In the preparative ultracentrifuge most of the fraction floated at  $d\ 1.063$  having apparently acquired the flotation characteristics of beta lipoprotein. In summary by the Cohn fractionation procedure, alpha lipoprotein was/

was increased but it neither showed its normal electrophoretic or ultracentrifugal characteristics, behaving as beta lipoprotein in these respects.

Fraction I and III showed the following changes. The cholesterol:phospholipid ratio was markedly reduced often to a level characteristic of fraction IV, V, VI. The ratio of free: ester cholesterol was markedly increased though not to the same extent as in the other fraction. These changes were reverted to normal with relief of jaundice. The fraction otherwise exhibited the usual characteristics of beta lipoprotein migrating typically on paper and floating at  $d$  1.063.

The atypical lipoproteins were further examined by Russ et al (1956) in a single case of xanthomatous biliary cirrhosis. Analysis of the Cohn fractions in a density gradient confirmed that the lipoproteins in fractions IV + V, and VI was concentrated into a density range close to that of beta lipoprotein./

lipoprotein. In the analytical ultracentrifuge  $S_f$  13 species predominated in fraction IV + V, and  $S_f$  25,  $S_f$  29 and  $S_f$  16 in fraction VI while fraction I + III was almost entirely  $S_f$  5.

Although the lipoproteins in the Cohn fractions, which normally contain alpha lipoprotein, behaved as beta lipoprotein in many respects, they failed to react with an anti-beta lipoprotein antiserum. Russ et al (1956) suggested that either the abnormal lipoprotein was antigenically dissimilar or that the antigenic site was blocked by the lipid (the ratio of peptide:lipid was reduced in all the fractions isolated). The anti-beta lipoprotein antiserum reacted normally with fraction I and III.

An antiserum prepared against aged high density lipoprotein was subsequently found by DeLalla et al (1957) to react with lipoprotein  $d < 1.063$  from the serum of the patient originally studied by Russ et al (1956); this antiserum did not react with normal low density lipoprotein. No conclusions were drawn by/

by DeLalla et al (1957) on the significance of their finding.

The occurrence of alpha lipoprotein of abnormal density in obstructive jaundice was suggested by Etienne et al (1966). Although they found alpha lipoprotein to be absent on paper electrophoresis, they observed an alpha lipoprotein precipitin arc of reduced mobility using immunoelectrophoresis. The abnormal fraction, as indicated by immunoelectrophoresis, was found within the density range 1.063 - 1.020 together with the beta lipoproteins.

A different lipoprotein abnormality in the serum of patients with obstructive jaundice was subsequently described by Switzer (1967). He found most of the lipid in such patients to be at d 1.019 - 1.063 and to show no reactivity with a potent anti-beta lipoprotein antiserum. Purified preparations of this material did not react with antisera to beta lipoprotein, alpha lipoprotein or normal/

normal human serum. It was rich in phospholipid and free cholesterol and low in protein. An antisera prepared against the abnormal fraction cross-reacted with normal  $d < 1.006$  lipoproteins. He suggested that this fraction represented an "obstructive jaundice lipoprotein" or "OLP" most closely resembling the VLDL fraction.

While this thesis was being prepared, Seidel et al (1969) claimed to have isolated 3 immunologically distinct lipoproteins LP-A, LP-B and LP-X from the LDL fraction ( $d 1.006 - 1.063$ ) of the plasma of patients with biliary obstruction. LP-A was defined as a lipoprotein characterised by presence of apolipoprotein A, and LP-B as lipoprotein characterised by presence of apolipoprotein B. LP-X had a characteristically high content of free cholesterol and phospholipid, resembled in these respects the abnormal lipoproteins described in Cohn fraction VI by Russ et al (1956) and the OLP of Switzer (1967) and was primarily responsible for the/

the unusual protein and lipid content of the LDL fraction. It was speculated that LP-X might be intestinal lipoprotein, similar to the C lipoprotein described by Gustafson et al (1966), normally rapidly catabolised in the liver and so largely undetectable in normal plasma, but accumulating in the plasma when liver derangement led to a decrease in its degradation rate. Screening tests in patients with various forms of jaundice showed LP-X to be limited to those subjects with an obstructive lesion.

SECTION II

INVESTIGATION

OBJECT OF INVESTIGATION

Since the various operational methods of defining the plasma lipoproteins fail to provide a totally consistent account of the alterations in the lipoprotein in subjects with various forms of obstructive jaundice, the present study was conceived. Its aim was to reinvestigate the lipid and lipoprotein changes in such subjects, especially by exploiting the immunochemical specificity of alpha and beta lipoproteins to permit their definitive identification.

Special objects of the study were -

- (a) the resolution of the conflicting evidence on the changes in alpha lipoprotein;
- (b) whether a true hyperbetalipoproteinaemia occurs;
- (c) the quantitative factors determining the altered behaviour of the lipoproteins;
- (d) whether subjects with biliary cirrhosis differ/

differ from those with other forms  
of obstructive jaundice in their  
lipid and lipoprotein abnormalities.

CHAPTER 3

SUBJECTS AND METHODS

SUBJECTS

Twenty-one patients with jaundice due to extra- or intra-hepatic cholestasis, or biliary cirrhosis were studied. Eighteen were under the care of various physicians (see Acknowledgements) who provided the plasma samples and the relevant clinical and laboratory findings. The other 3 patients attended the Clinical Center of the National Institutes of Health. Table 3.1 sets out the diagnosis given for each patient with the age and sex. The patients' serum total bilirubin and alkaline phosphatase levels at the time of sampling are given in Table 3.2 and were elevated in every case; the raised enzyme levels fell into the range associated with obstructive jaundice. In all but 1 case, the cause of the jaundice had been established; the exception was subject no. 9 in whom the diagnosis of intra-hepatic cholestasis was based on the biochemical features and the absence of extra-hepatic biliary obstruction as assessed/

assessed during surgery. Subjects no. 14, 16 and 20 were serially sampled during and after the jaundiced phase.

Blood samples were also obtained from 3 male and 2 female apparently healthy subjects whose ages ranged from 27 - 35 years. The main purpose of these control samples was to monitor the completeness of the ultracentrifugal separations rather than to provide quantitative control data. The samples were handled similarly to those of the jaundiced subjects and one was included with each batch of patient samples. In contrast to the findings which are to be described in some of the patients, the LDL fraction of the control subjects was found to contain beta lipoprotein only. When limited or no data was available for the normal values of a given parameter, the control data was especially used for comparison.

### METHODS

Blood was obtained from each subject in the morning after an overnight fast. It was anticoagulated with 0.1% EDTA to a concentration of 1 mg/ml. The plasma was obtained following centrifugation at 4° and stored at this temperature.

Aliquots of the plasma were taken for determination of total cholesterol, high density lipoprotein cholesterol, triglyceride and phospholipid levels and for the relative amounts of free to ester cholesterol. Lipoprotein electrophoresis on paper and immunoelectrophoretic analysis in agar and agarose were also performed on the plasma. The various methods employed are described below.

#### Isolation of Lipoprotein Fractions

Preparative ultracentrifugation: Plasma lipoprotein fractions were separated using the preparative ultracentrifuge by the method of Havel et al (1955).

5 ml of plasma was added to each of 2 cellulose nitrate ( $\frac{1}{2}$ " x  $2\frac{1}{2}$ " ) tubes. The plasma was layered with/

with 1.5 ml of 0.01 M EDTA - 0.15 M Na Cl solution and ultracentrifuged at  $10^0$  in the 40.3 rotor of a Spinco Model L preparative ultracentrifuge at 39,000 rpm (ca. 100,000 g) for at least 16 hours. The tubes were then sliced 2.1 cm below the lower rim of their caps. The top layer ( $d < 1.006$  fraction) from each tube was combined and the volume made up to 5 ml with EDTA-saline. The bottom layers ( $d > 1.006$  fraction) from the 2 tubes were also combined and restored to a volume of 10 ml using EDTA-saline. Aliquots of both fractions were taken for cholesterol determination, paper electrophoresis and immunoelectrophoresis. Of the balance of the  $d > 1.006$  fractions 5 ml was allocated to 1 cellulose nitrate tube and 4 ml to another. To the tube containing 5 ml was added 1 ml of a mixed salt solution of  $d 1.35$  (153 G Na Cl + 354 G KBr/L) producing a mixture of  $d 1.063$  which was layered with a solution of  $d 1.063$  (10 ml EDTA-saline of  $d 1.006$  + 2 ml Na Cl + KBr solution of  $d 1.35$ )/

d 1.35).

To the tube containing 4 ml was added 1 ml of EDTA-saline and thereafter it was brought up to d 1.063 as described for the first tube. They were then ultracentrifuged under similar conditions as the whole plasma. The tubes were sliced; the bottom fractions (d > 1.063) from each tube were combined and made up to a volume of 10 ml with EDTA-saline. Aliquots were taken for lipid determinations and for dialysis prior to electrophoresis. The top fraction (d 1.006 - 1.063) from each tube was recovered and transferred to a further tube, which was filled with the Na Cl KBr solution of d 1.063 and ultracentrifuged a second time. The tube was sliced and the bottom fraction discarded. The top fraction from each tube was kept separate and brought to a volume of 5 ml.

The LDL fraction which had been obtained from the 5 ml of the original d > 1.006 fraction, so that the lipoprotein was at plasma concentration, was used/

used for quantification of lipid and protein concentrations.

The LDL fraction which had been obtained from the 4 ml of the original  $d > 1.006$  fraction was dialysed at  $4^{\circ}$  against at least 100 volumes of 0.15 M Na Cl containing 0.01 M EDTA. The dialysis fluid was changed 3 times during the 16 hour period. This fraction was subsequently used for the various immunochemical and electrophoretic procedures.

**Cholesterol Determination:** Plasma cholesterol levels were determined following direct extraction of the plasma lipids by isopropanol (1 vol plasma: 20 vol isopropanol). The cholesterol levels in the LDL or HDL fractions were determined following extraction of the lipids by 25 vol of chloroform: methanol (2 vol:1 vol) to which was added 5 vol acidified water (concentrated  $H_2SO_4:H_2O$  2000 vol: 1 vol). The chloroform phase was recovered, appropriate aliquots were taken to dryness, and the lipids/

lipids redissolved in 2 ml isopropanol for the cholesterol determination. High density lipoprotein cholesterol levels in the plasma were determined in the supernate obtained following precipitation of all lipoproteins other than HDL by the addition of 0.15 ml 0.1 M manganese chloride and 6 mg of sodium heparin (Burstein and Samaille, 1960) to 3 ml of plasma. The supernate was extracted with chloroform:methanol as described above, and the cholesterol determination was performed in an isopropanol solution.

Cholesterol levels were determined using Autoanalyser method N-24b in which an anhydrous colour reagent containing ferric chloride in a mixture of acetic and sulphuric acids reacts with cholesterol and develops a colour which is read at 550 mu.

Triglyceride Determination: Triglyceride levels were determined by the semi-automated fluorometric procedure of Kessler and Lederer (1965) in which manually prepared isopropanol extracts of the plasma  
or/

or the LDL fraction were treated with a zeolite mixture (200 G zeolite, 20 G Lloyd Reagent, 10 G  $\text{CuSO}_4 \cdot 10 \text{H}_2\text{O}$ , 20 G  $\text{Ca}(\text{OH})_2$ ) to remove phospholipids, glucose, bilirubin and other chromogens. The automated system saponifies the triglyceride with KOH to glycerol, which is then oxidised to formaldehyde with periodic acid. The formaldehyde is condensed with diacetylacetone and ammonia (Hantzsch condensation reaction) yielding the fluorescent product 3, 5 - diacetyl - 1, 4 dihydro-lutidine. A blank determination on the isopropanol extract of each plasma was carried out by omitting the saponification step. The result obtained from this sample was used to correct for possible contamination from bilirubin which had not been removed from the icteric plasma by the zeolite mixture.

Phospholipid Determination: Phospholipid levels in the plasma, LDL and HDL fractions were determined by a modification of the method of Bartlett (1959). Prior extraction of the plasma or the lipoproteins was/

was carried out with chloroform:methanol in the usual manner. Aliquots of the chloroform phases were taken to dryness and digested with 0.1 ml concentrated  $H_2SO_4$  in a tube heater at  $180^\circ$  for one hour. The samples were cooled slightly and 4 drops of 30%  $H_2O_2$  were added. The samples were reheated for 30 minutes until they were clear; if necessary additional  $H_2O_2$  was added and the step repeated. To each tube was then added 2.3 ml 0.22% ammonium molybdate and 0.1 ml aminonaphthol sulfonic acid solution (0.25 G 1 - amino - 2 naphthol - 4 - sulfonic acid was added to 100 ml freshly prepared 15% sodium bisulfite solution to which was added 0.5 G sodium sulfite. It was stood overnight at  $4^\circ$ , filtered and stored at  $4^\circ$  in a dark bottle. It was prepared weekly.). Following thorough mixing, the tubes were heated in boiling water for 7 minutes and cooled to room temperature. The optical density was then read at 830 mu. A factor of 25 was used to convert the results for the lipid/

lipid phosphorus concentration into phospholipid concentration.

**Protein Determination:** The protein concentration of the LDL fraction was measured by the method of Lowry et al (1951); to 0.4 ml of the LDL fraction was added 2.0 ml of an alkaline copper solution (50 ml 2%  $\text{Na}_2\text{CO}_3$  in 0.10 N Na OH + 1 ml 0.5%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1% sodium or potassium tartrate). After 10 minutes 0.2 ml of Folin-Ciocalteu phenol reagent was added, and after 30 minutes the optical density was read in a Coleman colorimeter at 660 mu. A bovine albumen standard was used.

**Thin Layer Chromatography:** The relative amounts of free cholesterol to cholesterol ester in chloroform extracts of the plasma or of lipoprotein bands from paper electrophoretic strips was determined using thin layer chromatography (Stahl). Commercially available pre-coated 20 cm x 20 cm silica gel plates were used (Brinkman). The solvent system used was petroleum ether:diethyl ether:glacial acetic acid/

acid (90:10:1). After development of the plates the free and ester cholesterol spots were located using iodine vapour. They were scraped off the plate and the lipid was extracted in chloroform, taken to dryness and redissolved in isopropanol for cholesterol determinations.

The same solvent system was employed for separation of non-polar lipids before and after absorption of the LDL fraction with various antisera as described below; for polar lipids the solvent system used was chloroform:methanol:water (195:75:12). Both the non-polar and polar plates were sprayed with a mixture of glacial acetic acid: concentrated sulphuric acid: anisaldehyde (97:2:1), dried in an oven at 110<sup>o</sup> for 10 minutes, immediately inspected with the naked eye and photographed.

The amounts of lecithin, sphingomyelin and lysolecithin expressed as a percentage of the total lipid phosphorus recovered from each plate was determined/

determined in eluates of paper electrophoretic strips using two-dimensional thin-layer chromatography. The solvent systems used were chloroform:methanol:concentrated ammonium hydroxide:water (180:105:7.5:7.5) for the first dimension, and chloroform:methanol:concentrated ammonium hydroxide:water (120:160:5:5) for the second dimension. The spots were located using iodine vapour, scraped off the plate, and their lipid extracted with chloroform. The chloroform was evaporated and the lipid was redissolved in a given volume of chloroform and its phospholipid content determined.

#### Immunochemistry

Immuno-electrophoresis: Immuno-electrophoretic analysis was carried out on the plasma or lipoprotein fractions, following the method of Grabar and Williams (1955) adapted for microscope slides by Scheidegger (1955). Commercially pre-cleaned standard slides were first coated with a thin film of 2% agarose or 1% difco special noble agar in 0.05 M barbital buffer (pH 8.2)/

(pH 8.2). Two ml of buffered agarose or agar was then applied uniformly to each slide and allowed to gel. The plates were used shortly after preparation. Appropriate wells and troughs were cut out, and 2-3 ul of the plasma or the lipoprotein fractions was then put in a well and electrophoresed in 0.05 M barbital buffer (pH 8.2) at constant current (58 ma for agar; 44 ma for agarose) for exactly 40 minutes. The relevant antisera were added to the troughs and the slides were stored at room temperature for 24 hours in a high humidity chamber. They were then inspected for immunoprecipitin arcs, and photographed. The slides were washed overnight in saline and distilled water, dried and stained for lipid by immersion in a saturated solution of Oil red O in 60% ethanol at 40° for 12 hours.

Immunodiffusion: Immunodiffusion studies were carried out in Ouchterlony (1958) plates prepared with 1% difco special noble agar in saline. They were/

were developed at 4° and were inspected for precipitin lines and photographed at 24, 48 and 72 hours. All immunoprecipitin bands or lines held to represent lipoprotein were subsequently shown to stain for lipid with Oil red O.

Antisera: A variety of antisera were used. All but 2 of them had been previously raised in the Laboratory of Molecular Diseases against various antigens and were made available to the author. Details of the procedures used for their production are given by Levy and Fredrickson (1965). The species in which the antisera had been raised and the known reactivity of the antisera are set out in Table 3.3. Two of the antisera (360, Hyland anti-beta) had anti-beta lipoprotein reactivity only. All but one (ZAZ2) of the remaining antisera were known to react with alpha and beta lipoprotein; some of these also had reactivity against other plasma proteins. The anti-beta lipoprotein reactivity of antisera R1-R5 varied and was sometimes weak. Antiserum ZAZ2, which had been prepared/

prepared in rabbits using plasma from an abetalipoproteinaemic subject as the antigen, had anti-alpha lipoprotein but no anti-beta lipoprotein reactivity. It also reacted with pre-beta lipoprotein.

Gamma Globulin Preparation: Gamma globulin preparations of antisera 360 and 124A were prepared as described by Kekwick (1940). To each 100 ml of serum was added 18 G anhydrous  $\text{Na}_2\text{SO}_4$ . The solution was warmed to  $30^\circ$  for 2 hours. It was then centrifuged at  $25^\circ$  providing a fairly clear supernate which was discarded. 10 ml of water was added and (observed volume -10) ml was taken as the volume of the precipitate. This solution was made up to 40 ml and  $\text{Na}_2\text{SO}_4$  added to a concentration of 12%. It was warmed, stood and centrifuged as before, giving a clear supernate. To the precipitate was again added 10 ml of water to determine its volume, and the mixture was taken to a volume of 20 ml and a 12%  $\text{Na}_2\text{SO}_4$  solution was again produced. After warming and standing, a quite clear supernate was obtained/

obtained. The precipitate, which consisted of gamma globulin, was taken up in a small volume of 0.15 M saline and dialysed against 0.15 M saline.

Lipoprotein Paper Electrophoresis: Electro-  
phoretic analysis of plasma and of the ultracentrifugal fractions was performed on Whatman No. 1 paper (thickness 3 mm) by the Durrum hang-strip method in Beckman model R cells with barbital buffer (ionic strength 0.1, pH 8.6) containing 0.001 M EDTA and 1% albumen according to the method of Lees and Hatch (1963) except that bovine rather than human albumen was used. For electrophoresis of plasma or of the fractions  $d > 1.006$ ,  $d = 1.006 - 1.063$  and  $d > 1.063$ , 20  $\mu$ l samples were applied to each strip; for the fraction  $d < 1.006$ , 40  $\mu$ l samples were applied except when indicated in the text. The samples were run for 16 hours at constant voltage (120 v) producing a current of 0.75 - 1.0 ma per strip. The strips were dried for 20 minutes at 95<sup>0</sup>, immersed in a supersaturated solution of/

of Oil red O for 4 - 6 hours at 40<sup>o</sup>, rinsed and then dried.

The Oil red O was prepared by refluxing, in a round-bottomed flask 1.5 L of ethyl alcohol, 1.0 L of water, and 1 G of Oil red O. After the mixture had boiled it was allowed to cool and was then stored at 37 - 40<sup>o</sup> (Fredrickson et al, 1967).

In specified instances samples of LDL fractions were run in albumen-free buffer and corresponding strips stained for lipid with Oil red O and protein with bromophenol blue.

The strips to be stained for protein were immersed, for 2 hours, in 0.01% bromophenol blue in a 5% zinc sulphate and 5% acetic acid solution. They were then washed twice for 3 - 5 minutes in a 2% acetic acid solution, then for 5 minutes in a sodium acetate-acetic acid solution and dried.

Partial Delipidation of LDL Fraction: A slight modification of the method of Avigan (1957) was employed/

employed in which fresh (peroxide-free) diethyl ether was equilibrated for 20 mins in ice with an equal volume of diluted  $\text{NH}_4\text{OH}$  (1 drop  $\text{NH}_4\text{OH}$  to 100 ml distilled  $\text{H}_2\text{O}$ ). 0.2 ml of the LDL fraction was shaken with 50 ml of the ether for 4 - 6 hrs at  $4^\circ$ . The ether phase was removed by suction, the remaining solvent being evaporated with a nitrogen stream. The delipidated lipoprotein fraction in the remaining aqueous phase was used without delay for the immunochemical studies.

Details of other procedures are provided in certain subsequent chapters.

CHAPTER 4

IMMUNOCHEMICAL IDENTIFICATION OF ALPHA  
LIPOPROTEIN OF ABNORMALLY LOW DENSITY  
AND ALTERED ELECTROPHORETIC MOBILITY

Plasma lipoproteins show differing mobility during immunoelectrophoresis in agar or agarose gels (Fig. 4.1). In 2% agar, beta lipoprotein hardly leaves the origin, and pre-beta lipoprotein behaves similarly so that it cannot be distinguished (Levy et al, 1966). In 1% agarose, beta lipoprotein migrates with the mobility of a beta-globulin (Burstein and Fine, 1964, Levy et al, 1966) and pre-beta lipoprotein can be identified since it migrates between alpha and beta lipoprotein with  $\alpha_2$ -globulin mobility (Levy et al, 1966). Beta lipoprotein tends to react with agar and agarose gels, especially the former. In the present study agar gel (Fig. 4.2) was mainly used for immunoelectrophoretic analysis since, as indicated below, it proved to be the most satisfactory medium for demonstrating the abnormal precipitin arc found in some of the jaundiced subjects.

When plasma from each of the 21 icteric subjects was immunoelectrophoresed in 2% agar gel,

12 showed alpha and beta lipoprotein precipitin arcs only, the intensity of the former being diminished in several cases. An additional precipitin arc was present in the plasma of the other 9 subjects; this was a short, well-defined arc just peripheral to the beta lipoprotein arc and which had migrated slightly towards the cathode (Fig. 4.3). A dense material which had reacted with the agar gel, just internal to the new precipitin arc (Fig. 4.4a), was also often seen. The plasmas containing the additional arc were examined with a variety of anti-lipoprotein antisera. The new arc was observed with antisera with combined anti-alpha and anti-beta lipoprotein reactivity but not with monospecific anti-beta lipoprotein antisera; for example, in subject no. 14 it was produced with antisera R1, R2 and R5, 124A, alpha LpT<sub>1</sub> and 51. It was not produced by either of the two monospecific anti-beta lipoprotein antisera, 360 and Hyland anti-beta (Figs. 4.4a and 4.4b/

4.4b).

The various ultracentrifugal fractions were similarly examined for the new precipitin arc. It was not observed in the VLDL fraction nor, in particular, in the HDL fraction (Fig. 4.5) in which a normal alpha lipoprotein arc was visible. It was present in the  $d > 1.006$  fraction (Fig. 4.6) and was found to reside in the LDL fraction (Fig. 4.7).

Immunoelectrophoresis of the plasma and the LDL fraction in 1% agarose gel was also performed. It proved a less satisfactory medium for demonstrating the new precipitin arc which displayed an electrophoretic mobility similar to that of beta lipoprotein. Nevertheless, in some instances, an additional arc was clearly evident (Figs. 4.8 and 4.9).

The LDL fraction from the plasma of each of the subjects with the new precipitin arc was further examined by immunodiffusion in Ouchterlony plates. Such/

Such a procedure, in normal subjects, when appropriate antisera are used, results in the production of a single beta lipoprotein precipitin line and this was observed for 10 of the 12 subjects who had not shown the new arc on immunoelectrophoresis (Fig. 4.10). In the 9 subjects with the new arc, 2 lines were observed when the LDL fraction was diffused against anti-alpha and anti-beta lipoprotein antisera; a line giving a reaction of identity with the beta lipoprotein precipitin line either from a normal subject or those jaundiced subjects without the additional line, and a new precipitin line peripheral to the first line in relation to the centre well (Fig. 4.10). A single precipitin line showing identity with the normal beta lipoprotein line was found when a monovalent anti-beta lipoprotein antiserum was diffused against the LDL fraction (Fig. 4.10).

The LDL fraction was examined with a variety of antisera. As was the case for the immunoelectrophoretic/

electrophoretic findings, the new precipitin line was produced with several anti-alpha and anti-beta lipoprotein antisera, these being, for example, in subjects no. 14 and 15, 124A, RGA, R1, R5, alpha LpT<sub>1</sub>, alpha LpT<sub>2</sub>, AHS, and 79; the monovalent anti-beta lipoprotein antisera 360 and Hyland anti-beta each saw a single line only.

The abnormal immunoelectrophoretic and immunodiffusional findings just described were not obtained with all the antisera with anti-alpha lipoprotein reactivity.

It was possible, in several subjects, to study their plasma and LDL fractions during and after their jaundiced phase. In each instance the abnormal precipitin arc, which was present during the jaundiced phase, disappeared with the relief of the obstruction. For example, subject no. 16 was studied on 4 occasions (Fig. 4.11). On the first 3 (Mar. 7th, 11th and 20th) his plasma cholesterol was 526, 596 and 566 mg/100 ml respectively and his plasma phospholipid 1,155, 1,044 and 1,128 mg/100 ml. As Fig. 4.11 shows, the abnormal precipitin arc was present in each plasma, together/

together with normal beta lipoprotein and faint alpha lipoprotein precipitin arcs. By Mar. 29th, when the plasma cholesterol had fallen to 330 mg/100 ml and the phospholipid to 383 mg/100 ml, the abnormal arc was no longer present, there being only the normal beta and alpha lipoprotein arcs. Similarly the disappearance of the abnormal precipitin arc following relief of the jaundice was observed in the  $d > 1.006$  fraction (Fig. 4.6) and in the LDL fraction itself (Fig. 4.9).

Immunodiffusion studies in Ouchterlony plates gave similar results. In Fig. 4.12 as described above, the LDL fraction of subject no. 14 (Dec. 14th), when she was jaundiced with a plasma cholesterol of 640 mg/100 ml and a plasma phospholipid of 1,062 mg/100 ml, showed the additional precipitin line with several antisera with anti-alpha and anti-beta lipoprotein reactivity. This line was no longer evident in the LDL fraction from the sample of Feb. 2nd when the jaundice had been relieved, the plasma cholesterol had fallen to 288 mg/100 ml and the plasma phospholipid to 371 mg/100 ml. The fraction/

fraction now only showed the expected single beta lipoprotein precipitin line. Similar findings are seen in Fig. 4.10.

The additional precipitin line observed in the immunodiffusion studies was further characterized by examination of the LDL fractions before and after absorption with several antisera. 0.1 ml of the LDL fraction was reacted for 72 hrs at 4° with 0.2 ml or 0.3 ml of antiserum 124A (anti-alpha and anti-beta lipoprotein) or the monovalent anti-beta lipoprotein antisera 360 or Hyland anti-beta. The supernates were recovered and immunodiffused against a variety of antisera. In addition two- or three-fold dilutions of the LDL fractions were similarly treated. In Fig. 4.13 typical findings are shown. The saline-diluted LDL fractions still produced two precipitin lines following diffusion against antiserum R5 with anti-alpha and anti-beta lipoprotein reactivity. The supernates from the LDL fraction which had been/

been reacted with the monovalent anti-beta lipoprotein antiserum showed removal of the inner precipitin line, which had previously been shown to give a reaction of identity with normal beta lipoprotein (Fig. 4.11); the additional precipitin line had not been removed by the anti-beta lipoprotein antiserum. The supernates from the fraction reacted with 0.3 ml of the anti-alpha and anti-beta lipoprotein antiserum showed removal of the beta lipoprotein and the new precipitin lines.

The LDL fraction, containing the abnormal precipitin arc or line, was able to absorb the anti-alpha lipoprotein reactivity from a polyvalent antiserum. The 4 LDL fractions of the plasma of subject no. 16, obtained at various stages during and after his jaundice, were each reacted with an equal volume of polyvalent antiserum 81 with anti-alpha lipoprotein reactivity for 72 hrs at 4<sup>o</sup>. The supernates were obtained and immunodiffused against HDL prepared from a normal subject. The antiserum/

antiserum itself and a saline dilution (1:1 vol:vol) of the antisera were similarly studied. In Fig. 4.14 it is seen that the undiluted and diluted antiserum reacted with the normal HDL preparation to produce an alpha lipoprotein precipitin line. The supernates from the antisera reacted with the LDL fractions of Mar. 3rd and 7th no longer showed this anti-alpha lipoprotein reactivity and only a faint line is evident relating to the preparation of Mar. 11th. On the other hand the supernate of the antiserum treated with the LDL fraction of plasma taken on Mar. 29th when the jaundice was no longer present, when the plasma lipids had fallen substantially and the abnormal precipitin lines were absent, preserved its anti-alpha lipoprotein reactivity.

As subsequently discussed, it is thought that the additional precipitin arc or line observed in these various studies is a product of an over-lipidated alpha lipoprotein of abnormally low density/

density. Accordingly the effect of partial delipidation of the LDL fraction was studied. The LDL fractions were partially delipidated using cold ether as described in Chapter 3 and the ether was removed. The delipidated fractions were promptly examined by immunodiffusion; the abnormal line, peripheral to the beta lipoprotein precipitin line, was found to have disappeared and a new line was seen between the beta lipoprotein line and the central well. This line gave a reaction of partial identity with a heptane-delipidated HDL preparation from a normal subject (Fig. 4.15).

Ether delipidation of the LDL fraction was also found, on immunoelectrophoretic analysis, to remove the abnormal precipitin arc and to produce a lipoprotein precipitin arc with a mobility approximately equal to normal alpha lipoprotein (Fig. 4.16).

The results of these various immunochemical investigations are summarised and discussed in Chapter 6.

CHAPTER 5

PAPER ELECTROPHORETIC STUDIES:

DEMONSTRATION OF A LOW DENSITY LIPOPROTEIN

WITH UNUSUAL STAINING PROPERTIES

The immunochemical studies described in Chapter 4 show that, in certain subjects with obstructive jaundice or biliary cirrhosis, there occurs an alpha lipoprotein form of abnormally low density and changed electrophoretic mobility in agar and agarose gels, and with an altered rate of diffusion in agar gel in an Ouchterlony plate.

This chapter mainly describes the identification, by paper electrophoresis, of an additional lipoprotein band, which showed unusual staining properties, within the LDL fraction of these subjects.

Paper Electrophoresis of Plasma: The fasting plasma of each of the 9 subjects with the additional lipoprotein within the LDL fraction showed, on paper electrophoresis, a normal or slightly intense beta lipoprotein band (Fig. 5.1). In 5 of the subjects (nos. 15, 16, 18, 19 and 21) this band tended to be widened and indistinct (Fig. 5.1), resembling the "broad beta" band of Type III hyperlipoproteinaemia (Fredrickson et al, 1967). Since the presence of additional lipoprotein in the LDL fraction of these subjects prevented the quantitation of the beta lipoprotein within this fraction, a comparison of the intensity of the beta lipoprotein bands with beta lipoprotein levels could not be made.

A distinct pre-beta lipoprotein band was absent from the plasma of all but 1 (no. 14) of the subjects (Fig. 5.1), although they each had a triglyceride level which exceeded that in which a pre-beta/

pre-beta lipoprotein band usually occurs (80 mg/100 ml). For example, subject no. 16 had a tri-glyceride level of 600 mg/100 ml in his plasma sample of Mar. 7th but no pre-beta band.

The alpha lipoprotein band was reduced in intensity or absent in the electropherogram of the plasma (Fig. 5.1) of every subject except 1 (no. 17) whose band appeared normal. This is in keeping with the low alpha lipoprotein cholesterol and phospholipid levels of the subjects as described in Chapter 10.

The typical electropherogram of the plasma of these subjects therefore consisted of a beta lipoprotein band of normal or slightly increased intensity, sometimes broadened and indistinct, with a reduced or absent alpha lipoprotein band and an absent pre-beta lipoprotein band. Chylomicrons were not seen.

VLDL Fraction: A lipoprotein band of beta mobility was present in the VLDL fraction of 4 of the/

the 9 subjects and "trail" was frequently present (Fig. 5.2). The significance of this "floating" beta lipoprotein which was also observed for other subjects, is discussed in Chapter 12.

HDL Fraction: An alpha lipoprotein band was absent or only faintly present in the HDL fraction of all but 1 subject (no. 17) (Fig. 5.3) compatible with the appearances produced by their plasma.

LDL Fraction: The LDL fraction of all but 1 (no. 15) of the 9 subjects showed, with the Oil red O stain, a single band of beta mobility (Fig. 5.4). The beta lipoprotein bands were quite sharp and of normal width even in the 5 subjects whose plasma had shown a "broad" beta band, suggesting that this abnormal plasma band was due to lipoproteins not contained within the LDL fraction.

The LDL fractions were also electrophoresed in barbital buffer to which albumen had not been/

been added to permit the use of a protein stain. Corresponding strips were stained for protein with bromophenol blue, and for lipid with Oil red 0. In all subjects except no. 15 there was a protein band corresponding to the beta lipoprotein band stained by Oil red 0, and an abnormal band which had migrated to a point between the beta band and the origin (Figs. 5.5a and 5.5b). Occasionally this additional band was faintly visualised with Oil red 0 (Figs. 5.5a and 5.5b).

The presence of the abnormal band appeared to be related to the coincident jaundice since it was not present in those samples taken from subjects no. 14 and 16 after the jaundice had been relieved. For example Figs. 5.5a and 5.5b show the appearances in the LDL fraction of subject 16; 2 bands are present in each of the 3 fractions obtained when he was icteric but only a beta band is present in the 4th sample obtained when his jaundice had disappeared.

Subject/

Subject no. 15 showed 2 bands with either stain; a beta lipoprotein band and a cathodal migrating lipoprotein band (Fig. 5.6).

Although the abnormal band, which was designated the "slow" band, in the LDL fraction of the Group III subjects other than no. 15, did not usually stain for lipid with Oil red O, it was subsequently shown to contain lipid. For example, the LDL fraction of subject no. 16 (Mar. 11th) was electrophoresed on 6 paper strips (40  $\mu$ l/strip) simultaneously in a single cell. The 2 outer strips were stained for protein with bromophenol blue to locate the slow and beta lipoprotein bands. Using these strips as guidelines the portions of the remaining 4 strips corresponding to each of the bands were removed, cut into small pieces and pooled. The pooled material from each band was extracted with 10 ml chloroform:methanol (2:1 vol:vol) for 16 hrs, the chloroform phase was recovered after the addition of/

of 2 ml of acidified water, and was taken to dryness. The material so obtained was redissolved in a small volume of chloroform and chromatographed in thin-layer silica gel G plates in one dimension using the non-polar and polar solvent systems described in Chapter 3. The spots were stained with anisaldehyde. Non-polar and polar lipids were found in the samples derived from the slow band. The non-polar lipid from this band consisted mainly of free cholesterol, with faint traces of cholesterol ester and triglyceride (Fig. 5.7). The most prominent polar lipid was lecithin although a fainter sphingomyelin spot was also seen (Fig. 5.8). The beta lipoprotein band also produced non-polar spots corresponding to free and ester cholesterol, and triglyceride (Fig. 5.9) and polar spots corresponding to lecithin and sphingomyelin (Fig. 5.8) although the latter spot was less prominent than the corresponding spot from the slow band. Preliminary measurements of/

of the relative amounts of the various lipids present in the slower and beta bands of 2 subjects were carried out. The ratio of free cholesterol:cholesterol ester in each of the 2 bands was determined; six paper strips were electrophoresed as described above, the slow and beta bands located, and the lipid extracted with chloroform-methanol and taken into the chloroform phase. Half of this phase was ultimately chromatographed using a non-polar solvent system. The free and ester cholesterol spots were located with iodine vapour. Each spot was scraped off the plate and extracted with 50 ml chloroform. The chloroform was taken to dryness and the lipid redissolved in isopropanol and cholesterol concentration was determined. The results of this analysis which were carried out for subject no. 16 (Mar. 7th and 11th) and subject no. 18, are shown in Table 5.1.

The percentage of the total cholesterol present/

present as free cholesterol in each of the 3 slow bands was high, ranging from 79 - 87%. The beta band of subject no. 16, on each occasion showed a smaller but still considerable amount of its total cholesterol to be in the free form namely 81% (Mar. 7th) and 70% (Mar. 11th); that of subject no. 16 showed only 36% to be free. Of the total cholesterol in the LDL fraction of subject no. 16 (samples of Mar. 7th and Mar. 11th) 86% and 82% respectively was free cholesterol (Table 5.1). This is very close to the corresponding figures for their total plasma cholesterol, of which 85% and 88% was free (Table 10.4).

The percentage of the total LDL cholesterol which was in the slow band ranged from 59 - 77% in the 3 analysis for these 2 subjects (Table 5.1).

The levels of the individual phospholipids in the slow and beta lipoprotein bands of subject no. 16 were also measured; the lipids were eluted from the paper strips and developed, in 2 dimensions, by/

by thin-layer chromatography in silica gel G as described in Chapter 3.

Lecithin (Table 5.2) was the predominant lipid in the slow and beta bands constituting 74.1% and 79.3% respectively of the total phospholipid in each band. The slow band contained rather more sphingomyelin (14.9%) than the beta band (10.9%) but lysolecithin was present in approximately equal amounts. Of the total LDL phospholipid, 47% was present in the slow band.

CHAPTER 6

DISCUSSION

Immunochemical Findings in Low Density Fraction:

The observations described in Chapter 4 show that, in a high proportion of subjects with obstructive jaundice due to various causes, or biliary cirrhosis, an abnormal lipoprotein appears within their LDL fraction, in addition to beta lipoprotein. This abnormal lipoprotein was identified as a form of alpha lipoprotein on the basis of the immunochemical studies which are summarised as follows.

1. On immunoelectrophoresis in agar gel the LDL fraction of the subjects was found to contain a normal beta lipoprotein precipitin arc, and an abnormal cathodal-migrating lipoprotein precipitin arc when antisera with combined anti-alpha and anti-beta lipoprotein reactivity were employed. This abnormal arc was not obtained with either of 2 monovalent anti-beta lipoprotein antisera. Immunoelectrophoresis in agarose gel also demonstrated an additional precipitin arc of mobility closely/

closely similar to that of beta lipoprotein.

2. On immunodiffusion, the LDL fraction produced an additional precipitin line, peripheral to the beta lipoprotein precipitin line, with antisera with anti-alpha lipoprotein reactivity, but not with those with anti-beta lipoprotein reactivity only.

3. The additional precipitin line, and that representing beta lipoprotein, seen on immunodiffusion were not found in the supernate of the LDL fraction which had previously been reacted with an excess of antiserum with anti-alpha and anti-beta lipoprotein reactivity. Similar treatment of the LDL fraction with monospecific anti-beta lipoprotein antiserum led to the removal of the beta lipoprotein precipitin line only.

4. The LDL fraction was capable of absorbing the anti-alpha lipoprotein reactivity from an antiserum.

5./

5. Ether-delipidation of the LDL fraction led to the disappearance of the abnormal precipitin line seen on immunodiffusion and the production of a new line, internal to the beta precipitin line, and giving a reaction of partial identity with alpha lipoprotein. Delipidation also led to the disappearance of the additional arc seen during agarose gel immunoelectrophoresis, and the appearance of an arc with the approximate mobility of alpha lipoprotein.

These findings strongly suggest that the abnormal lipoprotein constituent of the LDL fraction is a form of alpha lipoprotein.

Paper Electrophoresis of Low Density Fraction:

In Chapter 5, the LDL fraction of the same subjects was shown, on paper electrophoresis, to contain an additional lipoprotein band which, although it did not usually stain with Oil red O, was found to contain lipid. It is/

is probable that this new lipoprotein band is the counterpart of the abnormal alpha lipoprotein form recognised in the LDL fraction by the immunochemical procedures. Confirmation of this assumption awaits the demonstration of the presence of alpha lipoprotein within the band.

Such a lipoprotein band has not been previously described though it may correspond to the additional component with slower mobility than beta lipoprotein reported by Kunkel and Ahrens (1949) during free electrophoresis of sera from 3 of 4 subjects with biliary cirrhosis and very high lipid levels.

The failure of the band to stain with Oil red O despite the presence of lipid requires explanation. An alcoholic solution of Oil red O stains fat since the Oil red O is more soluble in fat than in alcohol; non-polar lipids such as triglyceride take up the stain better than/

than polar lipid such as phospholipid (Chayen et al, 1969).

Theoretical models of alpha lipoprotein structure suggest that the polar phospholipids are situated at the surface of the complexes (Scanu, 1969) and that most of the non-polar lipid is confined to the interior. The lipid extracted from the slow band was shown, by thin-layer chromatography, to be predominantly lecithin and free cholesterol, the main lipids which have been found to accumulate in the plasma and in the various abnormal lipoprotein fractions which have been identified by other workers in the jaundiced subjects (Eder et al, 1955, Russ et al, 1956, Switzer, 1967, Seidel et al, 1969). It is possible that the excess phospholipid of the abnormal complex accumulates at the surface and prevents access of the Oil red O to the non-polar lipid within the interior.

Alpha Lipoproteins in Obstructive Jaundice:

Earlier/

Earlier observations on the alpha lipoproteins in obstructive jaundice, including biliary cirrhosis, suggested that they were diminished or absent. For example, Kunkel and Slater (1952), using zonal electrophoresis on potato starch, found the serum of 5 subjects with hyperlipidaemia and biliary cirrhosis, and of 2 subjects with extra-hepatic biliary obstruction, to have very low or absent alpha lipoprotein peaks. They commented on the apparently contrasting findings of Barr et al (1951) who found, in patients with biliary cirrhosis, an increase in the Cohn fraction carrying the alpha component of normal serum. To resolve this paradox they suggested that the high phospholipid content of the lipoproteins of the pathological sera, or their high bile salt levels, altered their solubility so that they were obtained in the Cohn fraction containing alpha lipoprotein.

As/

As briefly described in Chapter 2, the chemical findings were elaborated by Eder et al (1955) in a study of 7 patients with primary biliary cirrhosis, 5 with secondary biliary cirrhosis and 12 with bile duct obstruction, using Cohn method no. 10 (Cohn et al, 1950) for the fractionation of the plasma proteins. They found the increased plasma cholesterol in their subjects to be mainly present in Cohn fraction IV + V + VI which normally contains alpha lipoprotein. The phospholipids were proportionately increased so that the cholesterol:phospholipid ratio of the fraction was close to the normal of 1:2. Almost all of the cholesterol in the fraction was non-esterified. Similar changes were found in some subjects in the early obstructive stage of acute hepatitis.

Examination of Cohn fraction IV + V + VI by lipoprotein paper or starch electrophoresis showed/

showed all the lipids to migrate with the mobility of beta globulin. Poor correspondence was also observed between the percentage of total cholesterol and phospholipid in the  $d > 1.063$  fraction obtained by ultracentrifugation, and that in Cohn fraction IV + V + VI, there being much less lipid in the former. For example, in 3 subjects the  $d > 1.063$  fraction contained 3 - 11% of the total plasma cholesterol and 5 - 15% of the total phospholipid; corresponding figures for the Cohn fraction were 39 - 85% and 41 - 82%. These findings indicated that the greater part of the lipid in Cohn fraction IV + V + VI was of  $d < 1.063$ . It was concluded that an abnormal lipoprotein occurred in obstructive jaundice and speculated that either the abnormality consisted of the accumulation of lipoproteins which are normally present in small amounts, or that the lipid binding capacity of normal lipoproteins were altered, possibly/

possibly as a result of the accumulation of bile acids.

The abnormal lipoprotein with the Cohn fractions IV + V, and VI was isolated by Russ et al (1956); their findings are discussed in Chapters 2, and especially, in Chapter 8. They too showed the lipids of Cohn fractions which normally contained alpha lipoprotein to be unusual and to resemble in their preparative and analytical ultracentrifugal behaviour, density and electrophoretic mobility beta lipoprotein. Nevertheless, the fraction failed to react with an anti-beta lipoprotein antiserum.

Furman and Conrad (1957) using the analytical ultracentrifuge and employing a solvent density of 1.21 G/ml concluded that obstructive jaundice is characterised by reduced or absent HDL ( $d > 1.063$ ) of flotation rate  $-S_{1.21}^{0-20}$ .

The presence of alpha lipoprotein within the LDL fraction of subjects with obstructive jaundice was/

was implied by the work of DeLalla et al (1957). They prepared an anti-alpha lipoprotein antiserum in the rabbit using, as an antigen, aged acid-citrate-dextrose human plasma from which alpha lipoprotein was derived by ethanol precipitation and subsequent purification by ultracentrifugation. This antiserum reacted with the  $d < 1.063$  lipoprotein from the patient previously described by Russ et al (1956) but did not react with normal LDL.

Etienne et al (1966) reported the presence, on immunoelectrophoresis of serum from patients with complete biliary obstruction, of a lipoprotein fraction of only slight mobility ("mobile tres faible") most often migrating behind the beta lipoproteins and having the antigenic specificity of alpha lipoproteins, although no details of these properties were given. This abnormal fraction was found within the density range 1.020 - 1.063.

The/

The recognition, in the present study, of an alpha lipoprotein of unusually low density, decreased electrophoretic mobility and unusual staining properties explains the altered properties of the lipoproteins found by Eder et al (1955) and Russ et al (1956) within the Cohn fractions which normally contain alpha lipoprotein. Evidently the alterations in alpha lipoprotein responsible for its decreased density and the resultant alterations in its ultracentrifugal properties, and its reduced negative surface charge, do not displace it into the beta lipoprotein containing Cohn fractions.

Other Reports of Abnormal Low Density Lipoprotein: During or after the completion of the present work, 2 closely related papers appeared.

A new lipoprotein, the "obstructive jaundice/

jaundice lipoprotein" or "OLP" was reported by Switzer (1967) to be present in the LDL fraction of subjects with biliary obstruction. He studied an unspecified number of patients with surgically proven, complete biliary obstruction due to a variety of causes. Using a gamma globulin preparation of an antiserum against  $d < 1.063$  lipoproteins from normal fasting human subjects, he found the sera of some or all of these patients (this is not specified) to contain a large amount of non-precipitable  $d < 1.063$  lipoproteins. By comparison, the sera of subjects free of hepatic disease, including some with familial hypercholesterolaemia, contained only negligible amounts of unreactive  $d < 1.063$  lipoproteins. Immunoelectrophoretic and double diffusion studies, using a commercial antiserum to  $d < 1.063$  lipoproteins, confirmed the removal of all normal lipoprotein of  $d < 1.063$  by the antiserum.

The/

The non-reactivity of these  $d < 1.063$  lipoproteins did not seem to be explained, on the basis of various experiments, by the formation of soluble antigen-antibody complexes or by the interference with the antigen-antibody reaction by serum components such as conjugated bile acids.

Switzer (1967) prepared OLP by

- (a) precipitating reactive  $d < 1.063$  lipoproteins with the antiserum to  $d < 1.063$  lipoproteins.
- (b) recovering the unreactive  $d < 1.063$  lipoproteins by repeated precipitation with mepesulfate.
- (c) ultracentrifugation at  $d 1.063$ .

The amino-acid composition of this OLP preparation most nearly resembled that of the  $d < 1.006$  lipoproteins.

Rabbits were immunised with the OLP preparation. The resulting antisera reacted strongly with the OLP used for their preparation and with/

with freshly prepared OLP. It did not react with the  $d > 1.006$  fraction from normal serum but reacted, variably, with the  $d < 1.006$  fraction. A single, commercial antiserum to the  $d 1.063 - 1.21$  fraction (rabbit anti- $\alpha_1$  lipoprotein antiserum) and antisera to  $d < 1.063$  determinant did not react with the most purified preparations of OLP. He concluded that OLP contained an antigenic determinant distinct from that found in normal  $d 1.006 - 1.063$  lipoproteins but common to the one present in some  $d < 1.006$  fractions prepared from normal serum.

It may be asked whether the OLP of Switzer and the abnormal low density alpha lipoprotein observed in the present study are identical. The evidence offered by Switzer against OLP being alpha lipoprotein consists of (a) its amino-acid composition which most closely resembled that of  $d < 1.006$  lipoprotein. However, as he points out, the results were vitiated by the contamination/

contamination of the OLP by the gamma globulin preparation of the antiserum to the  $d < 1.063$  lipoprotein used in the preparation of the OLP.

(b) the failure of the single commercial anti-human  $\alpha_1$  lipoprotein antiserum to react with the purest OLP preparation. This point is discussed shortly.

Seidel et al (1969) have also described a new lipoprotein characterising obstructive jaundice which they designated "LP-X" (LP-X is defined by these workers as a lipoprotein occurring in obstructive jaundice and characterised by the presence of apolipoprotein X).

The new lipoprotein was isolated as follows. The  $d > 1.006$  fraction was acquired from plasma by preparative ultracentrifugation and precipitated with heparin. The precipitate was fractionated by the method of Cohn et al (1950); the fractions IV + VI containing LP-X. The behaviour of LP-X bore a close resemblance to that/

that of the low density alpha lipoprotein described here. It showed a cathodal mobility on immunoelectrophoresis in agar gel and an anodal mobility in agarose gel similar to that of the abnormal alpha lipoprotein. On paper electrophoresis it migrated just proximal to the beta lipoprotein band. A small amount of LP-A (lipoproteins characterised by the presence of apolipoprotein A) was also identified, by these workers, within the LDL fraction but, as measured in 2 subjects, it merely accounted for 2% of the total protein content of the fraction.

LP-X had a protein and lipid composition similar to the OLP of Switzer (1967) and the lipoprotein of Cohn fraction VI of the patient of Russ et al (1956).

It was also characterised immunochemically. Antisera to LP-X were prepared in rabbits. They did not react with normal HDL. Conversely, LP-X itself did not react with a monovalent anti-alpha<sub>1</sub> lipoprotein/

lipoprotein antiserum (Behringwerke, AG.) or an anti-whole human serum antiserum (Behringwerke, AG.).

It is likely that the abnormal lipoprotein arcs seen on agar and agarose gel immunoelectrophoresis by Seidel et al (1969) are identical to those observed in the present study, so that LP-X may be the abnormal form of alpha lipoprotein. If this is so, the failure of LP-X to react with anti-alpha lipoprotein antisera has to be accounted for.

One possibility is that Seidel et al (1969), like Switzer, used an insufficient variety of anti-alpha lipoprotein antisera to exclude the abnormal lipoprotein form being alpha lipoprotein. It is of interest that their commercial monovalent anti-alpha lipoprotein antiserum was derived from the same source as that of Switzer (1967). In the present study 10 antisera in which anti-alpha lipoprotein reactivity had been previously demonstrated were/

were employed and several did not consistently react with the abnormal lipoprotein. Recently, it has been shown that, contrary to previous findings, at least 2 major peptides can be identified in alpha lipoprotein, a newly described peptide characterised by C-terminal glutamine (R-Gln) and the previously described R-Thr (Shore and Shore, 1968). Small amounts of the 2 peptides recently found to be major constituents of VLDL, R-Ala and R-Val (Brown et al, 1969) have also been identified in HDL (Fredrickson, 1969). Each of these major or minor peptides may act as antigenic determinants and a given antiserum regarded as having anti-alpha lipoprotein reactivity may not react with all the determinants. Conceivably, the amounts of these various peptides relative to each other may be altered in the abnormal alpha lipoprotein form; it is subsequently suggested in Chapter 11 that this abnormal form results from overlipidation of/

of alpha lipoprotein, and since R-Gln may play a greater part in lipid binding than R-Thr (Gotto, 1969, Gotto and Shore, 1969). The former peptide may be especially present in the abnormal form.

The excess of lipid in the abnormal alpha lipoprotein may also mask some of the antigenic determinants. It is held, for example, that the failure of native VLDL to react with anti-alpha lipoprotein antisera is due to the large proportion of lipid in the molecular complex, since following partial delipidation anti-alpha lipoprotein antisera readily react with VLDL (Levy et al, 1966).

Support for the view that the LP-X is alpha lipoprotein with altered electrophoretic mobility also comes from Etienne et al (1966) who, as described above, found a precipitin line on immunoelectrophoresis, using anti-alpha lipoprotein antiserum, with a mobility approximating to/

to the abnormal line reported by Seidel et al (1969) and in the present work.

Seidel et al (1969) suggest that LP-X is present in all subjects with obstructive jaundice. They found it in the plasma of 38 patients in whom obstructive jaundice had been demonstrated by various other tests, but not in 23 patients in whom the causes of jaundice were non-obstructive.

The present findings differ in that only 9 of the 21 subjects showed an abnormal lipoprotein, its presence being related to the extent of the lipid and lipoprotein changes (see Chapter 10).

The relative amounts of beta and alpha lipoprotein within the LDL fraction of subjects nos. 13 - 21 was not determined in the present study. Preliminary results were obtained, however, for the percentage of the total LDL cholesterol or phospholipid transported in the slow band; it was/

was found to carry 59 - 77% of the cholesterol and 47% of the phospholipid (Tables 5.1 and 5.2). On the assumption that the slow band corresponds to the immunochemically identifiable low density alpha lipoprotein form, and that the latter is the equivalent of LP-X, then these figures are in broad agreement with the findings of Seidel et al (1969); they attributed 30 - 50% of the total serum cholesterol, and 50 - 80% of the total serum phospholipid to their LP-X fraction, most of the balance also being within the LDL fraction in LP-B. The present and their findings suggest that alpha lipoprotein is present in substantial amount, sometimes in excess of beta lipoprotein, in the LDL fraction.

CHAPTER 7

HYPERBETALIPOPROTEINAEMIA IN  
OBSTRUCTIVE JAUNDICE

This chapter presents evidence that, in 10 of the 21 subjects with obstructive jaundice or biliary cirrhosis, beta lipoprotein was the sole lipoprotein within the LDL fraction. Taken in conjunction with the findings in Chapter 10, it establishes that hyperbetalipoproteinaemia can occur in these disorders.

The plasma of subjects 1 - 10 was examined by lipoprotein paper electrophoresis. Six (no. 1, 2, 3, 8, 9 and 10) showed sharp lipoprotein bands of beta mobility and of above average intensity on visual inspection (Fig. 7.1), corresponding to their raised LDL cholesterol levels of 317, 309, 257, 257, 563 and 234 mg/100 ml respectively (Table 10.10). Three (no. 4, 5 and 6) showed beta lipoprotein bands of normal intensity (Fig. 7.1) and their LDL cholesterol level was normal or only slightly elevated at 209, 147 and 183 mg/100 ml respectively. The remaining subject (no. 7) had a weakly staining beta lipoprotein band and a beta lipoprotein cholesterol level of only 136 mg/100 ml. The beta lipoprotein bands were of normal width in all the subjects. A diminished alpha lipoprotein band was observed for each subject and pre-beta lipoprotein was usually absent.

On lipoprotein paper electrophoresis of the LDL fraction of subjects 1 - 10 a single band of beta/

beta mobility was observed. For example, Fig. 7.2 shows the findings in subjects no. 1, 2 and 3 and in a control subject. The sharp, intensely staining bands of the patients contrast with the normal band of the control subject. When albumen was omitted from the barbital buffer and corresponding strip stained for lipid with Oil red O and protein with bromophenol blue, a single protein band of identical mobility to the lipid staining band was seen (Fig. 7.3).

Immuno-electrophoretic analysis of the LDL fraction in agarose also gave findings consistent with beta lipoprotein being the sole lipoprotein in the fraction, when various anti-beta lipoprotein antisera, including several with anti-alpha lipoprotein reactivity, were employed. This is illustrated in Fig. 7.4 in which the polyvalent antisera 79 and 81, both with anti-alpha and anti-beta lipoprotein reactivity, have yielded a single sharp lipoprotein precipitin arc of beta mobility.

The/

The LDL fraction was also examined by immunodiffusion in Ouchterlony plates. When the fraction was diffused against a monovalent anti-beta lipoprotein antiserum such as 360 or an antiserum with anti-alpha and anti-beta lipoprotein reactivity such as 124A (Fig. 7.5), it consistently yielded a single precipitin line which gave a reaction of identity with a corresponding preparation from a control subject.

It was further shown that this line was no longer obtainable from the LDL fraction after it had been reacted with a monovalent or bivalent anti-beta lipoprotein antiserum. The procedure was as follows: 0.1 ml samples of each LDL fraction were reacted with three different volumes (0.03 ml, 0.08 ml, 0.10 ml) of a gamma-globulin preparation of antiserum 360 or 124A. Similar preparations were set up in which 0.1 ml EDTA-saline replaced the antisera. The preparations were stored at 4° for 72 hrs, centrifuged and the supernates obtained.

Samples/

Samples of these and of the unreacted LDL fraction were examined by immunodiffusion in agar gel against antisera 124A, 360, AHS and 79, all of which had known anti-beta lipoprotein reactivity (Fig. 7.6). The untreated and the EDTA-saline diluted LDL fractions from each subject produced a single beta lipoprotein precipitin line with each of the antisera. This line was absent from the LDL fractions which had been previously reacted with either of the 2 antisera, although the amount of antiserum required for its complete removal varied for different subjects, showing a direct relationship with the LDL cholesterol concentration.

The counterpart of these findings was obtained when the samples were examined by immunoelectrophoresis in agar gel; as shown in Fig. 7.7, a beta lipoprotein precipitin arc has resulted from the LDL fraction treated with EDTA-saline; its weaker intensity compared with the corresponding arcs obtained with native LDL (Fig. 9.5) is due to the four-fold/

four-fold dilution of the fraction. This arc is no longer found in the supernates from the LDL fractions which were reacted with either of the anti-beta lipoprotein antisera.

Lipoprotein paper electrophoresis also confirmed that the lipoprotein band of beta mobility observed within the LDL fraction was completely removed by appropriate amounts of antisera (Fig. 7.8).

A further series of experiments were designed to assess whether lipid, other than beta lipoprotein, could be identified with the LDL fraction employing the sensitive technique of thin-layer chromatography.

The relative volumes of the antisera required for complete removal of beta lipoprotein from the LDL fraction having been determined as described above, 0.3 ml of the LDL fraction was added to 0.3 ml or 0.5 ml of the gamma-globulin preparation of antiserum 124A or 360. Similar preparations of 0.3/

0.3 ml of the LDL fraction and 0.3 ml or 0.5 ml of EDTA-saline, and of 0.3 ml EDTA-saline and 0.3 ml or 0.5 ml of antiserum 124A or 360 were set up. The supernates were recovered and 0.4 ml of each was extracted with 10 ml chloroform:methanol (2:1 vol:vol); 2 ml of acidified water was added, the resulting phases were permitted to separate and the chloroform phase was recovered. 0.75 ml aliquots of this phase were taken to dryness, redissolved in a small volume of chloroform and analysed by one-dimensional thin-layer chromatography using polar or non-polar solvent systems. The plates were stained for lipid by anisaldehyde.

The samples derived from the saline-diluted LDL fractions showed, with the non-polar solvent system, spots corresponding to free and ester cholesterol, and triglyceride (Figs. 7.9a and 7.9b). With the polar system these samples produced lecithin and sphingomyelin spots and, to a lesser extent, phosphatidyl ethanolamine and phosphatidyl serine/

serine spots (Figs. 7.10a and 7.10b).

The corresponding samples derived from the LDL fractions which had been reacted with either of the antisera showed faint free and ester cholesterol, and phosphatidyl ethanolamine lipid spots only; these were similar in intensity to the spots obtained from the samples derived from the saline-diluted antisera, suggesting that the small amount of lipid observed in the supernate of the LDL fractions which had been reacted with the antisera originated from the antisera themselves. This interpretation is reinforced by the observation that these spots were also present in the antibody treated fraction from the control subject (Figs. 7.9b and 7.10b).

VLDL Fraction: As was described for some of the subjects described in Chapter 5, a lipoprotein band of beta mobility was observed on paper electrophoresis of the VLDL fraction of 6 of the 10 subjects. This finding is discussed in Chapter/

Chapter 12.

HDL Fraction: This fraction behaved normally on lipoprotein paper electrophoresis and on immunoelectrophoresis except that it appeared reduced in amount in keeping with the findings for the whole plasma.

CHAPTER 8

DISCUSSION

It is generally agreed that, in biliary cirrhosis and in different forms of obstructive jaundice, a characteristic disturbance in the plasma lipids occurs consisting of a marked increase in phospholipid and a less pronounced rise in total cholesterol, especially its esterified form. Triglyceride levels are only sometimes elevated and lipaemia does not occur (Man et al, 1945, Ahrens et al, 1950).

The application of electrophoretic and ultracentrifugal methods of analysis suggested that an increase in the beta lipoprotein fraction was partly or totally responsible for the elevated lipids. By means of free electrophoresis, Kunkel and Ahrens (1949) showed that a rise in beta globulin levels consistently occurred in patients with primary biliary cirrhosis. They found the area of the beta globulin peaks to be directly proportional to the total serum lipid concentration. Partial delipidation of the sera produced a considerable/

considerable reduction in the beta globulin components, in keeping with their being responsible for the transport of the excess lipid. Similarly Sterling and Ricketts (1949) studied 10 subjects with biliary cirrhosis which had developed following cholecystectomy for cholelithiasis. They showed, by free electrophoresis, that beta globulin levels were increased, sometimes strikingly so. They attributed this to an increase of a lipoprotein since they observed ether extraction of the sera to reduce the beta globulin peaks by about 66%.

Zonal electrophoresis, with starch as the supporting medium, was subsequently employed by Kunkel and Slater (1952), who found the sera of 5 subjects with biliary cirrhosis to show a very high peak with a mobility approximating to that of normal beta lipoprotein. Analysis of an extract of this peak showed it to consist predominantly of phospholipid. In discussing the compositional changes observed in the "beta lipoprotein" they suggested that/

that the lipoprotein was probably related to normal beta lipoprotein but had assumed a different composition; alternatively they postulated its being an entirely different lipoprotein of beta mobility.

Further evidence for an increase in beta lipoprotein was provided by McGinley et al (1952) using the analytical ultracentrifuge. They reported striking increases in the  $S_f$  6 and  $S_f$  8 lipoprotein classes and variable increases in the  $S_f$  10 - 17 classes.

Eder et al (1955) studied 12 patients with primary or secondary biliary cirrhosis, and a similar number with bile duct obstruction. Using lipoprotein paper electrophoresis they found almost all of the stainable lipid to migrate to the beta lipoprotein area, the alpha component being negligibly small. Flotation studies in the preparative ultracentrifuge of the plasma of 3 subjects with biliary cirrhosis correlated with the electrophoretic findings, in that only a small proportion of/

of the total serum cholesterol and phospholipid was in the fraction  $d > 1.063$ . A warning that conventional operational methods of defining the beta lipoproteins might be unreliable in these disorders was provided by their other observations. They fractionated the plasma proteins by Cohn method no 10 (Cohn et al, 1950) and found the increase in total plasma cholesterol to be due to its accumulation chiefly in Fraction IV + V + VI in which alpha lipoprotein is usually found. This fraction, however as studied in one subject, had the paper electrophoretic mobility of beta globulin, as did the lipoprotein of Fraction I + III which normally contains beta lipoprotein. Both fractions were of  $d < 1.063$ . The abnormal lipoproteins were also observed in the early stages of the jaundice of acute hepatitis when intrahepatic obstruction of finer biliary radicals are a possible feature (Watson and Hoffbauer, 1947).

These observations were extended, in a single subject/

subject with xanthomatous biliary cirrhosis, by Russ et al (1956). Having fractionated the plasma by Cohn method no 10 and examined the density gradients of the lipoproteins floated from the fractions, they found the lipids of Fraction IV + V to have the paper electrophoretic mobility of  $\beta_1$  and  $\beta_2$  globulins, to be concentrated in the density range 1.041 - 1.042, and to show compositional changes in the direction of a greatly reduced cholesterol: phospholipid ratio, a high free cholesterol: cholesterol ester ratio, and a low peptide:lipid ratio. Fraction VI also contained a lipoprotein which, on paper electrophoresis, dragged from the origin through the  $\beta_2$  globulin area. This fraction had 2 peaks of lipid concentration; one peak in the density range 1.035 - 1.038, and another in the range 1.039 - 1.049.

The major lipoprotein concentration of Fraction I + III appeared in the density gradient tube/

tube at 1.036 - 1.040, a slightly higher density range than normal, and its compositional features differed widely from normal beta lipoprotein. Nevertheless this fraction contained the antigenic determinant of beta lipoprotein giving precipitin reactions with antiserum against all the low density lipoproteins of normal plasma; the other Cohn fractions failed to react.

The flotation rates of each of the fractions were determined in the analytical ultracentrifuge. Fraction VI showed 3 species  $S_f$  25,  $S_f$  29 and  $S_f$  16. Fraction IV + V consisted of 2 species mainly  $S_f$  13 and a little  $S_f$  6. Fraction I + III almost entirely contained  $S_f$  5 molecules.

In summary on the basis of moving boundary and zonal electrophoresis, analytical and preparative ultracentrifugation, and Cohn fractionation, there is evidence that beta lipoprotein is increased in biliary cirrhosis and obstructive jaundice. That this evidence may be suspect is suggested by the anomalous/

anomalous reports just described, by other findings describing alpha lipoproteins (Etienne et al, 1966) or so-called "obstructive jaundice lipoprotein" or lipoprotein-X (Switzer, 1967, Seidel et al, 1969) within the LDL fraction as discussed in detail in Chapter 6, and by the evidence from the present study for the presence of alpha lipoprotein in the LDL fraction discussed in the same chapter. These various findings make it difficult to draw conclusions from the previous studies purporting to have demonstrated an increase in beta lipoprotein levels.

The findings of the present study offer strong evidence for the LDL fraction of the 10 patients described in the previous chapter consisting solely of beta lipoprotein. They are, in summary -

1. The LDL fraction migrated as a single band of beta globulin mobility on paper electrophoresis either when a lipid or a protein stain was employed.
2. The LDL fraction yielded a single lipid-staining precipitin arc of beta mobility when examined by immunoelectrophoretic/

immuno-electrophoretic analysis in agarose or agar gels using monovalent or polyvalent anti-beta lipoprotein antisera.

3. The LDL fraction produced a single lipid-staining precipitin line when immunodiffused against anti-beta lipoprotein antisera, and this line gave a reaction of identity with that obtained using normal beta lipoprotein.

4. The bands obtained on paper electrophoresis, and the precipitin arcs and lines observed on immuno-electrophoresis and immunodiffusion respectively, were no longer obtainable from the supernates of the LDL fractions which had been previously reacted with monovalent or polyvalent anti-beta lipoprotein antisera.

5. Examination of these supernates for lipid by a highly sensitive method (TLC) showed trace amounts only, and this could be attributed to the excess of the antisera.

Since the lipid within the LDL fraction of these/

these subjects appears exclusively in beta lipoprotein it is possible to assess whether this fraction is increased in concentration.

The LDL cholesterol concentration of each of the subjects is given in Table 10.10. The values may be compared with the age-related upper normal limits for LDL concentration in Table 10.1 which vary from 170 - 210 mg/100 ml. The use of beta lipoprotein cholesterol levels as a measure of beta lipoprotein concentration is based on the assumption that beta lipoprotein composition is fairly constant. Compositional changes were observed in the LDL fraction of the relevant jaundiced subjects (Tables 10.15 and 10.16, and see Chapter 10) but they were minor for cholesterol; this lipid contributed  $36.8 \pm 2.4\%$  by weight of the fraction of the 5 control subjects and  $32.4 \pm 4.8\%$  of that of the 9 jaundiced subjects, the difference being statistically/

statistically insignificant. Further, the percentage of cholesterol in the LDL fraction of normal subjects is usually taken somewhat higher (Table 1.2). Accordingly the cholesterol component of the LDL fraction of the jaundiced subjects tends to be low, so that the procedure for quantifying their beta lipoprotein generally underestimates the total beta lipoprotein concentration. The LDL cholesterol concentration of all but 2 (no. 5 and 7) of the 10 subjects fell beyond the corresponding upper 95% confidence limits, often substantially, and the mean value for the group was high at 261 mg/100 ml. It is, therefore, concluded that hyperbetalipoproteinaemia occurs in certain subjects with jaundice due to intra- or extra-hepatic obstruction or biliary cirrhosis. This hyperbetalipoproteinemia may be one of the several consequences of the lipid retention directly or indirectly due to intra- or extra-hepatic cholestasis; this is further discussed in Chapter 11.

CHAPTER 9

LOW DENSITY FORM OF ALPHA LIPOPROTEIN  
RETAINING NORMAL ELECTROPHORETIC MOBILITY

The identification of an alpha lipoprotein form, within the LDL fraction of 2 jaundiced subjects, is described in this chapter. Unlike the abnormal alpha lipoprotein of the subjects described in Chapter 5, it showed little or no alteration in its electrophoretic mobility.

On lipoprotein paper electrophoresis the whole plasma of subjects nos. 11 and 12 showed a prominent lipoprotein band with the approximate mobility of normal alpha lipoprotein, and a beta lipoprotein band (Fig. 9.1). The LDL fraction of both subjects produced, on lipoprotein paper electrophoresis, in addition to a beta lipoprotein band, a faintly staining band of alpha<sub>1</sub> mobility (Fig. 9.2). Both these LDL bands also stained for protein with bromophenol blue. The HDL fraction of each subject contained a prominent alpha lipoprotein band only (Fig. 9.3).

As was seen for many of the subjects in the other groups, the VLDL fraction of subject no. 11 contained a band of electrophoretic mobility similar to that of normal beta lipoprotein (Fig. 9.4). It was not observed in the VLDL fraction of subject no. 12.

The presence of alpha lipoprotein within the LDL/

LDL fraction of these subjects was confirmed on immunoelectrophoresis. For example, Fig. 9.5 shows the LDL fraction of subject no. 11 to contain 2 lipoprotein precipitin arcs; a normal beta lipoprotein arc, and a faint, faster moving arc of alpha mobility. These arcs were produced using antiserum 124A with anti-alpha and anti-beta lipoprotein reactivity. When a monovalent anti-beta lipoprotein antiserum such as 360 was used, the alpha lipoprotein precipitin arc was not seen, but only the beta lipoprotein arc.

Immunoelectrophoretic examination of the whole plasma and the other lipoprotein fractions revealed no other noteworthy features.

When the LDL fraction was examined by immunodiffusion in an Ouchterlony plate, it showed 2 precipitin lines against a variety of antisera which had, in common, anti-alpha and anti-beta lipoprotein reactivity (Fig. 9.6); an outer line which was shown to be due to beta lipoprotein, and/

and an additional inner line. The inner line was not removed from the LDL fraction by prior absorption with monovalent anti-beta lipoprotein antiserum 360. It disappeared following the reaction of the fraction with antiserum 124 with anti-alpha and anti-beta lipoprotein reactivity.

Unlike the additional precipitin line, also thought to represent alpha lipoprotein, seen in the LDL fraction of the subjects described in Chapter 4 (Fig. 4.13) it was internal to the beta lipoprotein line and not external.

The plasma lipid levels and lipoprotein composition of subjects no. 11 and 12 are described in Chapter 10 and the findings are discussed in Chapter 11. Here it is convenient to mention that the prominent alpha lipoprotein bands observed on paper electrophoresis were matched by the high HDL cholesterol levels (Table 10.10).

CHAPTER 10

QUANTITATIVE LIPID AND LIPOPROTEIN ALTERATIONS:  
CORRELATION WITH VARYING BEHAVIOUR  
OF ALPHA LIPOPROTEIN

The 21 patients with obstructive jaundice can be divided into 3 groups according to the behaviour of their alpha lipoprotein.

Group I in which, as described in Chapter 7, alpha lipoprotein retained its normal electrophoretic and ultracentrifugal characteristics, and in which hyperbetalipoproteinaemia was demonstrated.

Group II in which, as described in Chapter 8, some alpha lipoprotein was identified at  $d < 1.063$  but which showed little or no alteration in its electrophoretic mobility.

Group III in which, as described in Chapter 4, alpha lipoprotein was identified at  $d < 1.063$  and which showed abnormal electrophoretic mobility.

In an attempt to clarify the factors responsible for this varying behaviour of alpha lipoprotein, the plasma lipid and lipoprotein concentrations and aspects of the lipoprotein composition of these groups were studied and compared/

compared.

Plasma Lipids: The plasma cholesterol, triglyceride and phospholipid levels of each subject are shown in Table 10.2 and the mean values and standard deviations for each of the group in Table 10.3.

Age-related normal limits for cholesterol and triglyceride levels which had been previously established for the Laboratory of Molecular Disease (Fredrickson et al, 1967) are listed in Table 10.1, and were used for comparison. These limits are based on 95% fiducial limits calculated for small samples. The values for the 50 - 59 decade were also used for comparison with the results from jaundiced subjects aged 60 and over with respect to this and other data. Seventeen of the 21 subjects had cholesterol levels which fell above the upper normality limit, often considerably, the highest value being 800 mg/100 ml. The mean value for the whole series was 397/

397 mg/100 ml and was significantly higher than that of the 5 control subjects, as was the values for each of the 3 groups. In those subjects in whom lipid levels were serially determined, the cholesterol level was observed to fall following relief of jaundice (Table 10.4). No significant differences were noted between the mean cholesterol levels of each of the groups.

Triglyceride levels were elevated less frequently than cholesterol, exceeding the upper normality limit in 12 of the subjects. The mean value for the whole series was 200 mg/100 ml which did not differ significantly from that of the control subjects, and the highest value was 600 mg/100 ml. The levels fell in one of the subjects (no. 16) in whom jaundice was relieved (Table 10.4). None of the plasma samples were lipaemic, even in 3 subjects whose levels ranged from 378 - 642 mg/100 ml (Table 10.2/

10.2), levels at which lipaemia is normally apparent. On the contrary, the samples were quite clear.

Although the mean triglyceride level of each of the groups varied considerably, ranging from 96 mg/100 ml for Group II to 286 mg/100 ml for Group III, they did not differ significantly from one another.

Normal values for total plasma phospholipid levels have not been established for the Laboratory of Molecular Diseases. For a broad comparison the data of Adlersberg et al (1956) was employed; in Table 10.5 age- and sex-related upper normality limits for serum phospholipids were derived from this data by setting each upper limit at 2 standard deviations from the mean.

Of the 20 subjects in whom phospholipid levels were determined, 19 had elevated levels (Table 10.2). These elevations were sometimes striking/

striking; for example, in 3 subjects they exceeded 1,000 mg/100 ml and in 3 others, 600 mg/100 ml. The mean value for all 20 subjects was 556 mg/100 ml, and comparison with that of the 5 control subjects showed it to be significantly elevated. Similarly, the values of each of the groups was significantly elevated.

The hyperphospholipidaemia was reversible; in 2 subjects in whom the initial levels were 1,062 mg/100 ml and 1,155 mg/100 ml, the relief of the jaundice was associated with falls to 371 mg/100 ml and 383 mg/100 ml respectively (Table 10.4).

The mean phospholipid levels of the 3 groups differed considerably (Table 10.3), ranging from 393 mg/100 ml for the Group I subjects to exactly double for those of Group III. The higher level of the latter subjects differed significantly from that of the former, but not from that of the 2 Group II subjects who had identical levels of/

of 522 mg/100 ml. The mean level of the Group II subjects was not significantly higher than that of the Group I subjects.

Individual subjects in Group III showed considerable variation in their phospholipid levels (as reflected by the large standard deviation of 353 mg/100 ml) and several had values which overlapped those of Group I and Group II subjects.

Free Cholesterol and Cholesterol Ester: The ratio of free cholesterol:cholesterol ester in normal plasma is held within remarkably fine limits, shows minimal variations with age and sex (Goodman, 1965), and is usually taken as approximately 1:2. For the purposes of comparison with the findings in the jaundiced subjects, the normal values given by Eder et al (1955) were used in which the free cholesterol:cholesterol ester ratio ranges from 22:78 - 35:65. The 5 control subjects had ratios within this range/

range (Table 10.6). The results for the individual subjects are given in Table 10.6. Seven of the 10 Group I subjects, and all 8 of the Group III subjects in whom it was estimated showed a ratio increased beyond those limits. The ratio in each Group III subject was grossly disturbed to the extent that it was almost totally reversed. Of the 2 Group II subjects, one had a slightly increased ratio. There was no overlap between the ratios for the Group III subjects and those of other groups.

The mean values for the ratios of each of the groups are shown in Table 10.7. Those of Groups I and III were highly significantly elevated in comparison with that of the controls; that of Group II was not. The Group III mean was significantly higher than the mean for each of the other groups, which themselves did not significantly differ.

Normal values for plasma free cholesterol  
and/

and cholesterol levels were calculated from the age-related normal ranges of total cholesterol shown in Table 10.1 taking the free cholesterol:cholesterol ester ratio as 1:2, and are given in Table 10.8. The results for the control subjects were normal (Table 10.6).

The free cholesterol levels (Table 10.6) of 8 of the 10 Group I subjects were raised above the upper limit, but not to the same extent as the 8 Group III subjects in most of whom there was a striking rise. One of the 2 Group II subjects had a slightly elevated level. Each of the 3 groups had highly significantly elevated means in comparison to the controls (Table 10.9).

The mean free cholesterol level of Group III (Table 10.9) was considerably and significantly higher than that of Group I.

The cholesterol ester levels (Table 10.6)  
of/

of the individual subjects showed, for Group I a rise in 6 and a fall in 1, for Group II a rise in both, and for Group III a fall in 4. The higher mean level of the Group II subjects significantly differed from that of the controls.

The mean cholesterol ester level of the Group III subjects (Table 10.9) was significantly less than that of the Group II and Group I subjects.

Lipoprotein Quantification: The lipid and protein composition of each of the plasma lipoprotein fractions shows little variation both in health and in most hyperlipoproteinaemic states, so that changes in the concentration of a given lipoprotein fraction are paralleled by changes in the concentration of its individual lipids. Accordingly, Fredrickson et al (1967) introduced a system of quantifying the lipoprotein fractions by estimation, directly or indirectly, of the cholesterol concentration of each fraction. It involves estimation of/

- of A Plasma total cholesterol level -  
(LDL + VLDL + HDL) cholesterol level.
- B Cholesterol level of  $d > 1.006$  fraction =  
(LDL + HDL) cholesterol level.
- C HDL cholesterol level.

Then  $A - B =$  VLDL cholesterol level

$B - C =$  LDL cholesterol level

$C =$  HDL cholesterol level

Their normal values for LDL, VLDL and HDL cholesterol concentrations according to age, and in the case of HDL cholesterol also according to sex, are shown in Table 10.1.

LDL: The LDL cholesterol levels of all but 2 of the jaundiced subjects were elevated outside these limits; often considerably (Table 10.10). For example, subject no. 15 had a LDL cholesterol concentration of 579 mg/100 ml, the upper level of normal for a subject of his age being 190 mg/100 ml. However, comparison of the observed LDL cholesterol levels with those of normal subjects requires/

requires qualification. Firstly, as has been previously described and has been confirmed in the present study, compositional changes occur in the lipoproteins of these patients. These have the effect of reducing the relative contribution of cholesterol to the total LDL concentration and lead to an underestimation of the LDL concentration when it is measured in terms of the LDL cholesterol concentration. Secondly, in the subjects within Groups II and III the LDL fraction has been shown to contain alpha lipoprotein as well as beta lipoprotein. The percentage of cholesterol in alpha lipoprotein is normally considerably lower than in beta lipoprotein (Table 1.2). The compositional changes in the alpha lipoprotein of the jaundiced subjects variably alter this figure.

The most meaningful comparison is therefore between the subjects in Group I (Table 10.10), whose LDL fraction was shown, in Chapter 7, to contain/

contain beta lipoprotein only, and the normal subjects. Eight of the 10 patients in this group had elevated LDL cholesterol levels. Since their LDL cholesterol constituted, on average, 32.4% of the total LDL fraction which was lower, though not significantly, than the corresponding figure of 36.8% of the control group (Table 10.16), the degree of hyperbeta-lipoproteinaemia as indicated above was slightly greater than the figures for LDL cholesterol indicate. In addition, the mean value of this group was significantly higher than that of the controls.

Although the mean values for the LDL cholesterol of the groups varied considerably (Table 10.11), they were not significantly different. Such a comparison is valid since the mean percentage cholesterol content of the LDL fractions of the groups also did not differ significantly from one another (Table 10.16).

VLDL/

VLDL: Eleven subjects had elevated VLDL cholesterol levels, 2 had reduced levels and 7 had normal levels (Table 10.10). Despite a much higher mean level, Group III did not differ significantly from Group I or II (Table 10.11) nor did any of the means differ significantly from the controls.

HDL: The HDL cholesterol levels of the subjects in each group were estimated following either heparin-manganese precipitation of all other  $d < 1.063$  lipoproteins (Tables 10.10 and 10.11) or the ultracentrifugal separation of the  $d > 1.063$  fraction (Tables 10.13 and 10.14). The values obtained by either method correlated closely (for all subjects in the 3 groups  $r = 0.9125$ ;  $p < 0.001$ ).

With the heparin-manganese precipitation method (Tables 10.10 and 10.11), the mean value of the Group I subjects at 42 mg/100 ml was well within the range for normal subjects (Table 10.1);  
of/

of the 10 subjects in Group I the results were normal in 6, reduced in 3 and elevated in only 1. Of the Group II subjects 1 had an elevated level of 91 mg/100 ml and the other a level of 87 mg/100 ml near the upper limit. Eight Group III subjects had a mean value well below the lower normal limit; the levels of 6 fell into this category and only 2 had levels within the normal range. The higher value for the Group II subjects, and the reduced value for the Group III subjects differed significantly from that of the controls, and from one another (Table 10.11). The Group II value was also significantly higher than that of Group I.

There was some overlap between the values of the individual subjects of Groups I and III.

The results obtained for the HDL cholesterol concentrations of the groups following preparative ultracentrifugation (Tables 10.13 and 10.14) were similar both with respect to the mean values and/

and the statistical differences, except when Group I was compared with Group II or III.

HDL Phospholipid: The mean HDL phospholipid levels of each of the groups (Table 10.14) paralleled the changes in HDL cholesterol levels in that Group II had the highest level (213 mg/100 ml) and Group III the lowest (70 mg/100 ml). These values differed significantly one from the other (Table 10.13).

No published range of normal age-related HDL phospholipid levels was available for comparison but the value for the Group II subjects was significantly elevated over that of the controls.

Phospholipid:Cholesterol Ratio: Compositional changes in the HDL fraction were assessed by comparison of the phospholipid:cholesterol ratio (Tables 10.13 and 10.14). This ratio in normal HDL is slightly less than 2 (Table 1:2); the 5 control subjects had a mean ratio of 1.92. Each of the 3 groups had mean values considerably exceeding/

exceeding this, which differed highly significantly from that of the controls but not from one another.

LDL Composition: The percentage composition of the LDL fraction of the whole series of jaundiced subjects (Tables 10.15 and 10.16) was distinctly different from that of the 5 control subjects and was characterised by a significantly raised percentage of phospholipid and a significantly decreased percentage of cholesterol and protein. This pattern was also seen for the Group III subjects. In Groups I and II the percentage of LDL phospholipid was significantly raised, as was the percentage of protein in the former.

The mean LDL composition of the 3 groups of jaundiced subjects were compared with one another. Group III showed the greatest deviation from normal with 43.6% of the total non-hydrated weight consisting of phospholipid which differed significantly from/

from that of Group I and Group II, both of which had statistically similar mean levels of 32.8% and 34.0% respectively.

The subjects within Group III had the lowest mean LDL percentage protein content at 15.9%, significantly less than that of Group I (22.0%) and Group II (26.5%).

The LDL percentage cholesterol and triglyceride content of the groups were similar.

CHAPTER 11

DISCUSSION

Plasma Lipids: The changes in the plasma lipid levels of the jaundiced subjects conform closely to the pattern described by Man et al (1945) and Ahrens et al (1950) as outlined in Chapter 2. This lipid pattern, except when it occurs in the rare genetically determined disorder, plasma lecithin:cholesterol acyl transferase deficiency (Norum and Gjone, 1967), is exclusive to obstructive jaundice. Its main features, confirmed in Chapter 10, are the striking elevation in the levels of the plasma phospholipids, a less pronounced but fairly consistent increase in total plasma cholesterol levels, and the disruption of the normally stable relationship between the proportions of free and ester cholesterol (Goodman, 1965) in favour of an increase in the former. The changes in triglyceride levels, here measured directly in contrast to the indirect measurement of neutral fat by Man et al (1945), are less constant; nevertheless a majority of the subjects had/

had hypertriglyceridaemia. The reversibility of these various plasma lipid changes with the relief of the jaundice was also confirmed.

Hyperbetalipoproteinaemia: The study of the concentrations of the plasma lipoproteins described in Chapter 10 taken in conjunction with the findings in Chapter 7 established that, in almost half of the jaundiced subjects (Group I), beta lipoprotein concentrations were increased. Accordingly, obstructive jaundice can be numbered with disorders such as hypothyroidism, the nephrotic syndrome, multiple myelomatosis, idiopathic hypercalcaemia and acute porphyria as a cause of acquired Type II hyperlipoproteinaemia (Levy and Langer, 1969). Unlike the primary form and other secondary forms of hyperbetalipoproteinaemia, in which beta lipoprotein is increased in concentration but remains unaltered in composition, the elevated beta lipoprotein in obstructive jaundice displayed altered composition.

It/

It showed, in comparison with the LDL of the control subjects, in respect of its percentage composition, significantly increased phospholipid and decreased protein (Table 10.16). These changes reverted to normal with relief of jaundice. These compositional changes are in harmony with those described by Eder et al (1955) and Russ et al (1956) for the lipoproteins of Cohn fraction I and III. The LDL composition of the control subjects differs somewhat from that commonly quoted (Oncley and Harvie, 1969 and see Table 1.2) in which cholesterol, both free and ester, constitutes 43 - 45% of the total lipoprotein, and phospholipid and protein each about 22%. However, as discussed in Chapter 8, the differences are in the direction of magnifying the alterations from normal in the composition of the beta lipoprotein of the jaundiced subjects belonging to Group I.

Low/

Low Density Forms of Alpha Lipoprotein: The LDL (d 1.006 - 1.063) class normally contains beta lipoprotein only. Genetic variations (polymorphisms) of this normal beta lipoprotein have been described, namely the Ag system of Blumberg et al (1962) and the Lp system of Berg (1963). Rarely the LDL range contains lipoproteins other than normal beta lipoprotein. In subjects with abetalipoproteinaemia, who have a total deficiency of beta lipoprotein, there appears a low density lipoprotein with  $\alpha_1$  mobility on paper electrophoresis, and which has been further characterised as alpha lipoprotein on the basis of immunochemical studies and its total amino acid composition (Levy et al, 1966).

Recently, Sohdi (1969) detected, in the plasma of a healthy 10 year old boy with normal serum cholesterol and triglyceride levels, a lipoprotein of density between 1.040 and 1.063 with pre-beta mobility on paper electrophoresis and/

and producing an additional peak in the Schlieren pattern on analytical ultracentrifugation. This lipoprotein reacted with anti-LDL antiserum. The precise nature of this abnormal lipoprotein form remains to be fully characterised.

The present study furnishes 2 further examples of abnormal constituents of the LDL fraction, namely the low density alpha lipoprotein forms with altered (see Chapter 4) or virtually unaltered (see Chapter 9) electrophoretic mobility. The subjects with these changes were allocated, in Chapter 10, to Groups III and II respectively. Another group of subjects (Group I) was defined in terms of alpha lipoprotein being confined to its conventional density range and displaying normal electrophoretic mobility. The subjects of the 3 groups showed, as described in Chapter 9, various significant differences in the concentration of their/

their plasma lipids and lipoproteins, and in the composition of the latter.

The main statistically significant differences between the mean values of the groups were

- (1) The Group III subjects had higher phospholipid levels than those of Group I.
- (2) The Group III subjects had a higher free cholesterol level than the Group I subjects and a lower cholesterol ester level; and a higher free cholesterol: cholesterol ester ratio than those of Groups I and II.
- (3) The Group III subjects had the lowest HDL cholesterol (measured in the  $d > 1.063$  fraction) and phospholipid levels and Group I the highest. Similarly, the HDL cholesterol level of Group III (measured by heparin-manganese precipitation) was significantly higher than Group/

Group II, and that of Group II from Group I; however, the difference between Groups I and III just failed to achieve the 5 % significance level.

- (4) The Group III subjects had, in their LDL fraction, a lower percentage of protein and a higher percentage of phospholipid than each of the other groups.

It may therefore be asked whether the ultracentrifugal or electrophoretic alterations in the properties of alpha lipoprotein were determined by the changes in the lipoprotein concentrations and composition.

The separation of the plasma lipoproteins using the preparative ultracentrifuge is based on the different hydrated densities of the classes (Table 1.1). The hydrated density of a lipoprotein class is a function of its protein and lipid composition which is also characteristic for each class (Table 1.2). Since the density of/

of lipid varies from 0.9 - 1.0 G/ml, a roughly linear relationship exists between hydrated density and protein content (Lindgren and Nichols, 1960).

The alpha lipoprotein of the Group III and Group II subjects may have acquired a higher proportion of lipid than normally carried leading to a fall in its density to below 1.063. A degree of overlipidation of alpha lipoprotein insufficient to reduce its density to 1.063 might then explain the non-appearance of alpha lipoprotein in the LDL fraction of the Group I subjects.

Support for these concepts is obtained by the comparisons of the lipid and lipoprotein changes in the 3 groups, since as summarised above, they show the greatest degree of hyperlipidaemia (phospholipid, free cholesterol) to have occurred in the Group III subjects.

The significantly lower HDL cholesterol and phospholipid/

phospholipid levels of the Group III subjects in comparison to those of Group I could be explained by the conversion of most of their alpha lipoprotein into a lower density form, leaving only a small amount of alpha lipoprotein whose density, albeit probably lowered, still remained above 1.063.

The differences in the LDL composition of the Group III and I subjects, namely the greater proportion of phospholipid and the smaller proportion of protein can be interpreted as reflecting the entry, in the former group, of overlipidated alpha lipoprotein carrying an even higher proportion of phospholipid than it normally transports. The present study demonstrated a marked increase in the phospholipid:cholesterol ratio of the HDL fraction, especially of the Group III subjects compared with normal alpha lipoprotein (Table 10.14), and Eder et al (1955) and Russ et al (1956) previously observed similar changes in the/

the Cohn fractions which normally contain alpha lipoprotein. Although the composition of the beta lipoprotein within the LDL fraction of the Group III subjects could not be established, it is likely that, on account of the severer degree of hyperlipidaemia in these subjects, it was distorted along similar lines to, but to a greater extent than, the beta lipoprotein of the Group I subjects. The composition of the additional lipoprotein band found on paper electrophoresis of the LDL fraction of the Group III subjects and which, may represent the abnormal alpha lipoprotein, is in keeping with the above since the lipids predominating in it were lecithin and sphingomyelin, and free cholesterol.

An alternative explanation for the entry of alpha lipoprotein into the LDL range could be the formation of an abnormal complex between it and beta lipoprotein whose net density lay between 1.006/

1.006 - 1.063. No evidence of such complexing was found in the present study.

Electrophoretic Mobility of Abnormal Forms:

The variation in the electrophoretic mobility of the 2 forms of alpha lipoprotein requires explanation. Native alpha lipoprotein displays the electrophoretic mobility of an alpha<sub>1</sub> globulin. Evidence has been cited, in Chapter 1, for alpha lipoprotein being a constituent of pre-beta lipoprotein. The faster electrophoretic mobility of pre-beta lipoprotein compared with beta lipoprotein has been suggested to be due to the contribution, to the former, of the negative charge of alpha lipoprotein (Levy et al, 1966).

The abnormal low density alpha lipoprotein detected in the Group III subjects showed altered electrophoretic mobility both in agar and agarose gels. Its probable counterpart on paper electrophoresis also showed a mobility quite different from that of normal alpha lipoprotein. Presumably the net surface charge of the lipoprotein has been reduced/

reduced by the overlipidation of the molecule, especially by phospholipid; as has been outlined in Chapter 6, postulated molecular models of alpha lipoprotein place its normal complement of phospholipid at the surface of the complex. Alternatively the excess lipid may have led to conformational changes in the lipoprotein and alteration in its surface charge. A reduction in charge might also be related to the altered free cholesterol:cholesterol ester ratio (see below).

Group II Subjects: The changes in the lipoproteins in the 2 subjects belonging to Group II merit special comment. Like the other jaundiced subjects they showed hypercholesterolaemia and hyperphospholipidaemia. The degree of this hyperlipidaemia placed them in an intermediate position between the subjects of the other groups.

Unlike the other subjects their free cholesterol:cholesterol ester ratio was normal, and their/

their mean HDL cholesterol and phospholipid levels were significantly higher than those of Groups I and III and of the control subjects, in keeping with the unusually prominent alpha lipoprotein bands identified on paper electrophoresis of their plasma and HDL fraction.

These raised levels of HDL lipid may be due to a degree of hyperlipidaemia sufficient to overlipidate normal alpha lipoprotein, but insufficient to reduce the density of more than a fraction of the lipoprotein to below 1.063.

The normal electrophoretic mobility of this relatively small amount of alpha lipoprotein could then be attributed to an insufficient degree of overlipidation to significantly alter the net surface charge. The maintenance of a normal free cholesterol:cholesterol ester ratio by each of the 2 Group II subjects, despite an absolute increase in both components, is noteworthy. It raises the possibility that alterations in the electrophoretic/

electrophoretic mobility of alpha lipoprotein occur only when this ratio is increased.

Source of Excess Lipid: Several sources could account for the additional lipid which alters the composition of both the alpha and beta lipoproteins and increases the concentration of the latter. It may accumulate secondary to the biliary obstruction and the release from the liver of the retained free cholesterol and phospholipid. Phospholipid, mainly in the form of lecithin (Nakayama and Blondstrand, 1961) constitutes about 82% by weight of normal human bile and free cholesterol 9%. Other lipids are present in only small amounts (Nakayama and Johnston, 1962).

An additional or alternative source of lipid may result from enhanced hepatic synthesis; following the production of obstructive jaundice, in the rat, by bile duct ligation (Fredrickson et al, 1954) there occurs an increase in cholesterol synthesis/

synthesis in the intact animal or by liver slices or homogenates, an observation recently confirmed by Weis and Dietschy (1969) who found biliary ligation to produce an increment, of two and a half to three times, in the rate of hepatic cholesterolgenesis.

An alteration in the activity of the lecithin:cholesterol acyl transferase (LCAT) enzyme might contribute to the accumulation of free cholesterol and lecithin. This enzyme catalyses the transfer to an acyl radical from the 2 position of lecithin to the 3-hydroxyl of free cholesterol, the products of the reactions being lysolecithin and cholesterol ester (Glomset, 1968). Studies on several affected members of a kindred with an apparent deficiency of the enzyme suggest that it is responsible for the formation of most of the cholesterol ester of human serum (Norum and Gjone, 1967). The enzyme may be associated, in the plasma, with HDL/

HDL (Lossow et al, 1966) and it is possible that LCAT acts on lipid previously bound to HDL (Schumaker and Adams, 1969). It may be synthesised in the liver (Brot et al, 1962). Impaired activity of the enzyme which has been reported to occur in liver disease (Simon and Scheig, 1970) might be expected to result in accumulation of lecithin and free cholesterol, especially in HDL. However, this seems to occur predominantly in parenchymatous liver disease rather than in obstructive jaundice or biliary cirrhosis in which the enzyme activity has been observed to be normal or high (Simon and Scheig, 1970) although contrary findings have been previously reported (Turner et al, 1953).

Lipid Binding Properties of Alpha Lipoprotein:

The lipid-binding properties of alpha lipoprotein or its apoprotein indicate a potential for the overlipidation postulated to occur in the subjects in the present study.

The apoprotein of HDL has a strong affinity  
for/

for lipid, and HDL itself might be capable of binding more lipid than it normally carries, especially free cholesterol and phospholipid. For example, native HDL was shown to combine with a wide variety of lipid molecules when they were dispersed with Celite (Ashworth and Green, 1963). Much larger amounts of free sterol than sterol ester could be introduced. Scanu and Hughes (1960) and Scanu and Page (1961) labelled the apoprotein, obtained following almost complete delipidation of HDL, with  $^{131}\text{I}$  and showed that, when added to whole human serum, it yielded a labelled complex displaying the ultracentrifugal and electrophoretic characteristics of normal HDL. Subsequently Scanu (1967) showed human apoprotein A to recombine with aqueous dispersions of lecithin, phosphatidyl ethanolamine and sphingomyelin in an energy-free system suggesting non-covalent bonds between the protein and/

and phospholipid. The avidity of the protein for lipid seemed to reside in the primary structure of the former since it was retained even when the alpha helical structure of HDL had been completely unfolded by the presence of urea (Scanu, 1965).

A greater affinity of delipidated human LDL for phospholipid and free cholesterol than for triglyceride and cholesterol ester was found by Sohdi and Gould (1967). Control experiments with other plasma proteins showed no such lipid-binding suggesting that the affinity of apoHDL for phospholipid and cholesterol was a specific property of the apolipoprotein. This affinity for phospholipids may also be reflected by the difficulty with which they may be removed from alpha lipoprotein compared with other lipids in delipidation procedures (Fredrickson et al, 1967). The probable presence of HDL in chylomicrons may also indicate the/

the affinity of HDL for lipid (Fredrickson et al, 1967).

It has further been suggested that phospholipids have the property of stabilising lipoproteins so that their capacity for cholesterol binding is increased (Ahrens and Kunkel, 1949).

CHAPTER 12

"FLOATING" BETA LIPOPROTEIN  
IN OBSTRUCTIVE JAUNDICE

A lipoprotein of beta mobility on paper electrophoresis at  $d < 1.006$  was described for many of the jaundiced subjects. Chapter 12 discusses this finding and its possible cause.

A lipoprotein with beta mobility on paper electrophoresis was found in the  $d < 1.006$  fraction of subjects belonging to each of the 3 groups, as indicated in Chapters 5, 7 and 9. In this abnormal property it resembles the "floating" beta lipoprotein which defines familial Type III hyperlipoproteinaemia (Fredrickson et al, 1967). It was present in the VLDL fraction of 6 of the 10 Group I subjects, 1 of the 2 Group II subjects and 4 of the 9 Group III subjects. Only 3 of the subjects, all of Group III, showed the "broad beta" band usually, but not invariably, seen on lipoprotein paper electrophoresis of the whole plasma of subjects with familial Type III hyperlipoproteinaemia. Almost all the subjects, including those with raised triglyceride levels lacked a distinct pre-beta or a chylomicron band, suggesting that normal or excess levels of triglyceride were transported predominantly in this abnormally light beta lipoprotein form.

The/

The changes leading to this acquired form of "floating" beta lipoprotein can only be conjectured at this stage. In Type III hyperlipoproteinaemia the basic biochemical defect is unknown. In Tangier Disease or Familial Alpha Lipoprotein Deficiency (Fredrickson, 1966) a hereditary and complete deficiency of normal alpha lipoprotein is also associated with the presence of some beta-migrating lipoprotein at  $d < 1.006$ . Alpha lipoprotein, with beta lipoprotein, is a normal component of pre-beta lipoprotein (Levy et al, 1966) and may, by the contribution of its negative charge, be responsible for altering the mobility of the complex from beta to pre-beta. Its absence in Tangier Disease may determine an inability to form normal pre-beta lipoprotein complexes. Instead the VLDL fraction would contain a complex which retained beta mobility (Levy et al, 1966).

Although alpha lipoprotein was present, to an apparently variable extent, in all the jaundiced subjects/

subjects it varied in density and electrophoretic mobility in Groups II and III, and in all groups, in its lipid composition or concentration. It is possible that this abnormal alpha lipoprotein is incapable of participating in normal pre-beta lipoprotein complexes. Alternatively, it may be present in the pre-beta complex, but, because of a decreased negative charge or an altered structural relationship with the other components of the complex, it fails to confer on it a pre-beta mobility.

Resolution of this problem awaits more detailed studies of the VLDL fraction of the jaundiced subject.

## REFERENCES

REFERENCES

- Addison, T., Gull, W., Guy's Hosp. Rep. (1851),  
7, (ser 2), 265.
- Adlersberg, D., Schaefer, L.E., Steinberg, A.G.,  
Wang, Chun-I., J. Amer. Med. Ass. (1956), 162,  
619.
- Ahrens, E.H. Jr., Kunkel, H.G., J. exp. Med.  
(1949), 90, 409.
- Ahrens, E.H., Payne, M.A., Kunkel, H.G., Eisen-  
menger, W.J., Blondheim, S.H., Medicine (1950),  
29, 299.
- Ashworth, C.A.E., Green, C., Biochim. Biophys.  
Acta (1963), 70, 68.
- Avigan, J., J. biol. Chem. (1957), 226, 957.
- Barr, D.P., Russ, E.M., Eder, H.A., Amer. J. Med.  
(1951), 11, 480.
- Bartlett, G.R., J. biol. Chem. (1952), 234, 466.
- Berg, K., Acta path. microbiol. scand (1963),  
59, 369.
- Bierman/

- Bierman, E.L., Porte, D., O'Hara, D.D., Schwartz,  
M., Wood, F.C., J. clin. Invest. (1965), 44,  
261.
- Blumberg, B.S., Bernanke, D., Allison, A.C.,  
J. clin. Invest. (1962), 41, 1936.
- Brot, N., Lossow, W.J., Chaikoff, I.L., J. Lipid  
Res. (1962), 3, 413.
- Brown, W.V., Levy, R.I., Fredrickson, D.S.,  
J. biol. Chem. (1969), 244, 5687.
- Brown, W.V., Levy, R.I., Fredrickson, D.S.,  
Biochim. Biophys. Acta (1970), 200, 573.
- Burstein, M., Samaille, J., Clin. chim. Acta (1960),  
5, 609.
- Burstein, M., Fine, J.M., Rev. franc Et. clin. biol.  
(1964), 9, 420.
- Chayen, J., Bitensky, L., Butcher, R.G., Poulter,  
L.W. in A Guide to Practical Histochemistry,  
Oliver and Boyd, Edinburgh 1969, p 84.
- Cohn/

- Cohn, E.J., Gurd, F.R.N., Surgenor, D.M.,  
Barnes, B.A., Brown, R.K., Derouaux, G.,  
Gillespie, J.M., Kahnt, E.W., Lever, W.F.,  
Liu, C.H., Mittleman, D., Mouton, R.F.,  
Schmid, K., Uroma, E., J. Amer. chem. Soc.  
(1950), 72, 465.
- DeLalla, V., Gofman, J.W. in Methods of Bio-  
chemical Analysis, ed. D. Glick, Interscience,  
New York 1954, p 459.
- DeLalla, L., Levine, L., Brown, R.K., J. exp.  
Med. (1957), 106, 261.
- Dole, V.P., Hamlin, J.T., Physiol. Rev. (1962),  
42, 674.
- Eder, H.E., Russ, E.M., Rees Pritchett, R.A.,  
Wilber, M.M., Barr, D.P., J. clin. Invest. (1955),  
34, 1147.
- Epstein, F.H., Block, W.D., Proc. Soc. exp. Biol.  
(N.Y.) (1959), 101, 740.
- Etienne/

- Etienne, G., Etienne, J., Polonovski, J.,  
Housset, E., Cottet, J., Rev. Int. Hepat.  
(1966), 16, 169.
- Farquhar, J.W., Ways, P. in The Metabolic Basis  
Inherited Disease, 2nd Edition, ed. J.B.  
Stanbury, J.B. Wyngaarden, D.S. Fredrickson,  
McGraw Hill, New York 1966, p 509.
- Feigl, J., Biochem. Z. (1918), 86, 1.
- Flint, A. Jr., Amer. J. med. Sci. (1862), N.S.,  
44, 305.
- Frazer, A.C., Discussions of Faraday Soc. (1949),  
6, 81.
- Fredrickson, D.S., Loud, A.N., Hinkelman, B.T.,  
Schneider, H.S., Frantz, I.D., J. exp. Med.  
(1954), 99, 43.
- Fredrickson, D.S. in The Metabolic Basis of  
Inherited Disease, 2nd Edition, ed. J.B. Stanbury,  
J.B. Wyngaarden, D.S. Fredrickson, McGraw Hill,  
New York 1966, p 486.
- Fredrickson/

- Fredrickson, D.S., Levy, R.I., Lees, R.S.,  
New Eng. J. Med. (1967), 276, 32, 94, 148,  
215, 273.
- Furman, R.H., Conrad, L.L., J. clin. Invest.  
(1957), 36, 713.
- Gage, S.H., Cornell Vet. (1920), 10, 154.
- Gitlin, D., Cornwell, D.G., Nakasoto, D.,  
Oncley, J.L., Hughes, W.L., Janeway, C.A.,  
J. clin. Invest. (1958), 37, 172.
- Glomset, J., J. Lipid Res. (1968), 9, 155.
- Goodman, D.S., Physiol. Rev. (1965), 45, 747.
- Gotto, A.M., Levy, R.I., Fredrickson, D.S.,  
Proc. Nat. Acad. Sci. (U.S.) 1968, 60, 1436.
- Gotto, A.M., Proc. Nat. Acad. Sci. (U.S.) 1969,  
64, 1119.
- Gotto, A.M., Shore, B., Nature (1969), 224, 69.
- Grabar, P., Williams, C.A., Biochim. Biophys.  
Acta (1955), 17, 67.
- Greten/

- Greten, H., Levy, R.I., Fredrickson, D.S.,  
J. Lipid Res. (1969), 10, 326.
- Gustafson, A., Alaupovic, P., Furman, R.H.,  
Biochemistry (1966), 3, 632.
- Hatch, F.T., Moore, J.L., Lindgren, F.T., Jensen,  
F.T., Jensen, L.C., Freeman, N.K., Wills, R.D.,  
Circulation (1967), 36, II - 16.
- Havel, R.J., Eder, H.A., Bragdon, J.H., J. clin.  
Invest. (1955), 34, 1345.
- Havel, R.J. in Handbook of Physiology Section 5:  
Adipose Tissue, ed. A.E. Renold, G.F. Cahill,  
Waverley Press, Baltimore 1965, p 499.
- Kekwick, R.A., Biochem. J. (1940), 34, 1248.
- Kessler, G., Lederer, H. in Automation in Analy-  
tical Chemistry, ed. L.T. Skeggs, Medaid Incor-  
porated, New York 1965, p 341.
- Kunkel, H.G., Ahrens, E.H., J. clin. Invest. (1949),  
28, 1575.
- Kunkel, H.G., Slater, R.J., J. clin. Invest. (1952),  
31, 677.
- Lees/

Lees, R.S., Hatch, F.T., J. Lab. clin. Med. (1963),  
61, 518.

Levy, R.I., Fredrickson, D.S., J. clin. Invest.  
(1965), 44, 426.

Levy, R.I., Fredrickson, D.S., Laster, L.,  
J. clin. Invest. (1966), 45, 531.

Levy, R.I., Lees, R.S., Fredrickson, D.S.,  
J. clin. Invest. (1966), 45, 63.

Levy, R.I., Langer, T., Modern Treatment (1969),  
6, 1313.

Lindgren, F.T., Elliot, H.A., Gofman, J.W.,  
J. Phys. Chem. (1951), 55, 80.

Lindgren, F.T., Nichols, A.V. in The Plasma  
Lipoproteins vol. II, ed. F.W. Putnam, Academic  
Press, New York 1960, p 10.

Lossow, W.J., Shah, S.N., Chaikoff, I.L.V.,  
Biochim. Biophys. Acta (1966), 116, 172.

Lowry, O.H., Rosebrough, N.J., Farr, A.L.,  
Randall, R.T., J. biol. Chem. (1951), 193, 265.

Man/

Man, E., Kartin, B.L., Durlacher, S.H., Peters, J.P.,

J. clin. Invest. (1945), 24, 623.

Margolis, S. in Structural and Functional Aspects

of Lipoproteins in Living Systems, ed. E. Tria,

A.M. Scanu, Academic Press, London and New York

1969, p 369.

Marshall, W.E., Kummerow, F.A., Arch. Biochem. Biophys.

(1962), 98, 271.

McGinley, J., Jones, H., Gofman, J., J. invest. Derm.

(1952), 19, 71.

Nakayama, F., Blomstrand, R., Acta chem. scand.

(1961), 15, 1595.

Nakayama, F., Johnston, C.G., J. Lab. clin. Med.

(1962), 59, 364.

Nichols, A.V., Strisower, E.H., Lindgren, F.T.,

Adamson, G.L., Coggiola, E.L., Clin. chim. Acta

(1968), 20, 277.

Nichols, A.V., Proc. nat. Acad. Sci. (U.S.) (1969),

64, 1128.

Norum/

Norum, K.R., Gjone, E., Scand. J. clin. Lab. Invest.  
(1967), 20, 231.

Oncley, J.L., Harvie, N.R., Proc. nat. Acad. Sci.  
(U.S.) (1969), 64, 1107.

Ouchterlony, O. in Progress in Allergy, S. Karger,  
Basle 1958, p 5.

Pollard, H., Scanu, A.M., Taylor, E.W., Proc. nat.  
Acad. Sci. (U.S.) (1969), 64, 304.

Russ, E.M., Raymunt, J., Barr, D.P., J. clin.  
Invest. (1956), 35, 133.

Scanu, A.M., Page, I.H., J. exp. Med. (1959), 109,  
239.

Scanu, A.M., Hughes, W.L., J. biol. Chem. (1960),  
235, 2876.

Scanu, A.M., Page, I.H., J. Lipid Res. (1961), 2,  
161.

Scanu, A.M., Proc. nat. Acad. Sci. (U.S.) (1965),  
54, 1969.

Scanu, A.M., J. biol. Chem. (1967), 242, 711.

Scanu/

Scanu, A.M. in Structural and Functional Aspects of  
Lipoproteins in Living Systems, ed. E. Tria, E.M.  
Scanu, Academic Press, London and New York 1969,  
p 440.

Scheidegger, J.J., Int. Arch. Allergy (1955),  
7, 103.

Schumaker, V.N., Adams, G.H. in Annual Review of  
Biochemistry, Annual Reviews Inc., ed. E.E.  
Snell, Palo Alto 1969, p 114.

Seidel, D., Alaupovic, P., Furman, R.H., J. clin.  
Invest. (1969), 48, 1211.

Shore, B., Arch. Biochem. (1957), 71, 1.

Shore, B., Shore, V., Biochemistry (1968), 7, 3396.

Simon, J.B., Scheig, R., J. clin. Invest. (1970),  
283, 841.

Sohdi, H.S., Metabolism (1969), 18, 852.

Sohdi, H.S., Gould, R.G., J. biol. Chem. (1967),  
242, 205.

Sterling, K., Ricketts, W.E., J. clin. Invest. (1949),  
28, 1469.

Switzer/

- Switzer, S., J. clin. Invest. (1967), 11, 1855.
- Thannhauser, S.G. in Lipidoses. Diseases of the Cellular Lipid Metabolism, Oxford University Press, New York 1940.
- Turner, K.B., McCormack, G.H., Jr., Richards, A., J. clin. Invest. (1953), 32, 801.
- Watson, C.J., Hoffbauer, F.W., Ann. int. Med. (1947), 26, 813.
- Weis, H.J., Dietschy, J.M., J. clin. Invest. (1969), 48, 2398.
- Windmueller, H.G., Levy, R.I., J. biol. Chem. (1967), 242, 2246.

PLASMA LIPID AND LIPOPROTEIN CHANGES  
IN OBSTRUCTIVE JAUNDICE:  
IMMUNOCHEMICAL IDENTIFICATION OF ABNORMAL  
ALPHA LIPOPROTEIN FORMS

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CHAPTER 1

TABLE 1.1

OPERATIONAL CLASSIFICATIONS OF PLASMA LIPOPROTEINS

Paper Electrophoresis	Preparative Ultracentrifuge	Analytical Ultracentrifuge
Chylomicrons	Chylomicrons ( $d < 0.95$ )	$S_f^* > 400$
Beta	Low density (LDL) ( $d 1.006 - 1.063$ )	$S_f 0 - 20$
Pre-beta	Very low density (VLDL) ( $d 0.95 - 1.006$ )	$S_f 20 - 400$
Alpha	High density (HDL) ( $d 1.063 - 1.21$ )	Sediments at $d 1.063$

\*  $S_f$  unit = Svedberg unit of flotation expressed in  
 $10^{-13}$  cm/sec/dyne/G (in solution of  
NaCl of  $d 1.063$  and temperature  $26^{\circ}$ )

TABLE 1.2

TYPICAL LIPID AND PROTEIN COMPOSITION OF PLASMA LIPOPROTEINS

composition figures (after Oncley and Harvie, 1969) are

expressed as % of dry weight of each lipoprotein;

contribution of carbohydrate moiety is neglected

	Chylomicrons	Pre-beta Lipoprotein	Beta Lipoprotein	Alpha Lipoprotein
CHOLESTEROL	5	13	43	18
TRIGLYCERIDE	90	65	10	2
PHOSPHOLIPID	4	12	22	30
PROTEIN	1	10	25	50

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CHAPTER 3

TABLE 3.1

AGE, SEX AND DIAGNOSIS OF SUBJECTS STUDIED

Subject no.	Age	Sex	Diagnosis
1	32	M	biliary cirrhosis
2	39	M	post-necrotic cirrhosis progressing to intra- hepatic cholestasis
3	37	M	biliary cirrhosis
4	42	F	biliary cirrhosis
5	45	M	biliary cirrhosis
6	15	F	biliary cirrhosis
7	39	M	stone in common bile duct
8	48	M	stone in common bile duct
9	49	F	intrahepatic cholestasis
10	38	F	biliary cirrhosis
11	50	F	biliary cirrhosis
12	65	F	biliary cirrhosis
13	41	M	biliary cirrhosis
14/			

TABLE 3.1 (contd.)

14	51	F	carcinoma of pancreas
15	45	M	biliary cirrhosis
16	54	M	carcinoma of pancreas
17	61	M	carcinoma of pancreas
18	60	M	biliary cirrhosis
19	56	F	biliary cirrhosis
20	30	M	stone in common bile duct
21	46	M	Hodgkin's disease with glands in porta hepatis

TABLE 3.2

SERUM BILIRUBIN AND ALKALINE PHOSPHATASE LEVELS  
OF SUBJECTS STUDIED

Subject no.	Total bilirubin (mg/100 ml)	Alkaline phosphatase
		B = Bodansky units K-A = King-Armstrong units
1	8.9	12 B
2	4.2	48 K-A
3	3.8	24 B
4	4.8	80 K-A
5	2.3	96 K-A
6	1.2	88 K-A
7	2.0	38 K-A
8	8.0	74 K-A
9	2.9	22 B
10	8.4	80 K-A
11	9.2	66 K-A
12	2.3	67 K-A
13	13.8	34 B
14/		

TABLE 3.2 (contd.)

14	15.6	53 K-A
15	3.9	48 K-A
16	30.0	50 K-A
17	14.9	73 K-A
18	25.8	17 B
19	22.0	> 50 K-A
20	6.8	22 B
21	7.0	70 K-A

TABLE 3.3

REACTIVITIES OF ANTISERA USED IN IMMUNOCHEMICAL STUDY

Name	Species used to prepare antiserum	Beta Lipoprotein (lipid moiety)	Reactivity		
			Alpha Lipoprotein	Albumin	Other plasma proteins
360	sheep	+	-	-	-
Hyland anti- beta lipoprotein	goat	+	-	-	-
124	sheep	+	+	+	-
124A*	sheep	+	+	-	-
79	sheep	+	+	+	+
81	sheep	+	+	+	+
AHS	goat	+	+	+	+

ZAZ 2/

TABLE 3.3 (contd.)

ZAZ 2	rabbit	-	+	+-	VLDL
R1-R5	rabbit	+-	+	-	VLDL
Alpha L <sub>p</sub> T <sub>1</sub> and T <sub>2</sub>	rabbit	+	+	+	-
51	rabbit	+	+	+	-
BGA	rabbit	+	+	-	-

\* absorbed with excess albumin

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CHAPTER 5

TABLE 5.1

FREE AND ESTER CHOLESTEROL COMPOSITION OF "SLOW" AND  
 BETA LIPOPROTEIN BANDS FROM LOW DENSITY LIPOPROTEIN FRACTION

FC = free cholesterol      CE = cholesterol ester

Subject no.	FC	% of total cholesterol in band		% of total LDL cholesterol in Slow band	
		Slow	Beta		
16	(Mar. 7th)	FC	87	81	59
		CE	13	19	
18	(Mar. 11th)	FC	87	70	68
		CE	13	30	
		FC	79	36	77
		CE	21	64	

TABLE 5.2

PHOSPHOLIPID COMPOSITION OF "SLOW" AND  
BETA LIPOPROTEIN BANDS FROM LOW DENSITY  
LIPOPROTEIN FRACTION OF SUBJECT NO. 16

	% of total phospholipid in band	
	Slow	Beta
Lecithin	74.1	79.3
Sphingomyelin	14.7	10.9
Lysolecithin	5.2	4.4
Other phospholipids	6.1	4.4
% of total LDL phospholipid in Slow band	47	

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CHAPTER 10

TABLE 10.1

NORMAL LIMITS FOR PLASMA CHOLESTEROL, TRIGLYCERIDE AND LIPOPROTEIN CHOLESTEROL  
 CONCENTRATIONS EMPLOYED IN LABORATORY OF MOLECULAR DISEASES

lipid levels, in mg/100 ml

Age (years)	Cholesterol	Triglyceride	VLDL		LDL		HDL	
			Cholesterol	Cholesterol	Cholesterol	Cholesterol	Males	Females
0 - 19	120 - 230	10 - 140	5 - 25	50 - 170	30 - 65	30 - 70		
20 - 29	120 - 240	10 - 140	5 - 25	60 - 170	35 - 70	35 - 75		
30 - 39	140 - 270	10 - 150	5 - 35	70 - 190	30 - 65	35 - 80		
40 - 49	150 - 310	10 - 160	5 - 35	80 - 190	30 - 65	40 - 85		
50 - 59	160 - 330	10 - 190	10 - 40	80 - 210	30 - 65	35 - 85		

Sex differences are ignored except for HDL cholesterol concentrations

TABLE 10.2

PLASMA LIPID LEVELS OF INDIVIDUAL SUBJECTS WITH OBSTRUCTIVE JAUNDICE

results as mg/100 ml

	Subject no.	Cholesterol	Triglyceride	Phospholipid
Group I	1	370	141	481
	2	371	488	470
	3	304	155	355
	4	288	259	330
	5	183	152	291
	6	279	152	403
	7	174	197	209
Group II/	8	346	96	270
	9	668	51	723
	10	502	282	399

TABLE 10.2 (contd.)

Group II	11	360	138	522
	12	423	54	522
Group III	13	232	102	428
	14 (Dec. 14th)	640	298	1,062
	15	800	378	1,276
	16 (Mar. 7th)	526	600	1,155
	17	442	249	729
	18	462	190	-
	19	383	132	540
	20	260	100	337
	21	313	180	615

Controls (IV) //

TABLE 10.2 (contd.)

Controls (IV)	T	191	274	229
	M	154	73	203
	B	156	95	162
	L	192	80	210
	E	148	92	148

TABLE 10.5

PLASMA LIPID LEVELS IN GROUPS OF SUBJECTS WITH OBSTRUCTIVE JAUNDICE

results (mean and standard deviation) as mg/100 ml; figures in

brackets refer to number of subjects studied.

Group	Cholesterol	Triglyceride	Phospholipid
I	349 ± 147 (10)	197 ± 123 (10)	393 ± 144 (10)
II	392 ± 45 (2)	96 ± 59 (2)	522 ± 0 (2)
III	451 ± 184 (9)	286 ± 161 (9)	786 ± 353 (8)
I + II + III	397 ± 161 (21)	200 ± 153 (21)	556 ± 298 (20)
Controls (IV)	168 ± 21 (5)	123 ± 85 (5)	190 ± 34 (5)

Significances/

TABLE 10.3 (contd.)

Significances					
I - II	t	0.3956	1.063	1.2152	
	p	> 0.05	> 0.05	> 0.05	
I - III	t	1.3454	0.7704	3.0685	
	p	> 0.05	> 0.05	< <u>0.01</u>	
II - III	t	0.4358	1.2656	0.9412	
	p	> 0.05	> 0.05	> 0.05	
I - IV	t	2.6756	1.2069	3.0440	
	p	< <u>0.02</u>	> 0.05	< <u>0.01</u>	
II - IV	t	9.6442	0.3978	13.0144	
	p	< <u>0.001</u>	> 0.05	< <u>0.001</u>	
III - IV	t	3.3583	1.5934	3.5863	
	p	< <u>0.01</u>	> 0.05	< <u>0.005</u>	
(I + II + III)-IV	t	3.1167	1.3179	2.6955	
	p	< <u>0.05</u>	> 0.05	< <u>0.02</u>	

TABLE 10.4

PLASMA LIPID AND LIPOPROTEIN CHOLESTEROL LEVELS (mg/100 ml)

IN SUBJECTS DURING AND AFTER JAUNDICE

<u>Subject no. 14</u>	Dec. 14th	Dec. 27th	Feb. 2nd
jaundice	+	+	-
cholesterol	640	442	288
triglyceride	298	246	308
phospholipid	1,062	743	371
free cholesterol	512	314	133
cholesterol ester	128	128	155
free cholesterol: cholesterol ester	80:20	71:29	46:54
<u>lipoprotein cholesterol</u>			
VLDL	60	10	72
LDL	568	409	200
HDL (heparin-manganese precipitation)	12	23	16

Subject no. 16/

TABLE 10.4 (contd.)

<u>Subject no. 16</u>	Mar. 7th	Mar. 11th	Mar. 20th	Mar. 29th
jaundice	+	+	+	-
cholesterol	526	596	566	330
triglyceride	600	642	460	198
phospholipid	1,155	1,044	1,128	383
free cholesterol	447	524	481	125
cholesterol ester	79	72	85	205
free cholesterol: cholesterol ester	85:15	88:12	85:15	38:62
<u>lipoprotein cholesterol</u>				
VLDL	160	200	208	40
LDL	345	378	348	274
HDL (heparin-manganese precipitation)	21	18	10	16

TABLE 10.5

NORMAL UPPER LIMITS FOR SERUM PHOSPHOLIPID LEVELS

levels (mg/100 ml) calculated from data of Adlers-  
berg et al (1956) as mean + 2 standard deviations

Age (years)	Males	Females
13 - 17	186	192
18 - 22	209	210
23 - 27	214	214
28 - 32	259	209
33 - 37	243	216
38 - 42	259	234
43 - 47	248	253
48 - 52	250	268
53 - 57	255	303
58 - 62	254	293
62 - 67	276	-

TABLE 10.6

PLASMA FREE CHOLESTEROL AND CHOLESTEROL ESTER LEVELS  
OF INDIVIDUAL SUBJECTS WITH OBSTRUCTIVE JAUNDICE

Subject no.	free cholesterol		cholesterol ester	
	mg/100 ml	% of total cholesterol	mg/100 ml	% of total cholesterol
Group I				
1	167	45	204	55
2	197	53	174	47
3	109	36	195	64
4	115	40	173	60
5	71	39	112	61
6	89	32	190	68
7	82	47	92	53
8	118	34	228	66
9	234	35	434	65
10	256	51	246	49
Group II/				

TABLE 10.6 (contd.)

Group II	11	130	36	230	64
	12	110	26	313	74
Group III	13	167	72	65	28
	14 (Dec. 14th)	512	80	128	20
	15	640	80	160	20
	16 (Mar. 7th)	447	85	79	15
	17	305	69	137	31
	18	-	-	-	-
	19	310	81	73	19
	20	182	70	78	30
	21	213	68	100	32
Controls (IV)					

TABLE 10.6 (contd.)

Controls (IV)	T	59	31	132	69
	M	45	29	109	71
	B	51	33	105	67
	L	46	24	146	76
	E	60	34	117	66

TABLE 10.7

RELATIVE AMOUNTS OF PLASMA FREE AND ESTER CHOLESTEROL

IN GROUPS OF SUBJECTS WITH OBSTRUCTIVE JAUNDICE

results (mean and standard deviation) expressed as a

percentage of total plasma cholesterol

No. of subjects	Group I (10)	Group II (2)	Group III (8)	I + II + III (20)	Controls (IV) (5)
Free cholesterol	41 ± 7	31 ± 7	75 ± 7	54 ± 20	30 ± 4
Ester cholesterol	59 ± 7	69 ± 7	25 ± 7	46 ± 20	70 ± 4

Significances

t	I - II	I - III	II - III		
	1.7893	10.3057	8.5112		
p	> 0.05	< 0.001	< 0.001		
t	I - IV	II - IV	III - IV	(I + II + III) - IV	
	3.0753	0.2013	13.8387	2.6583	
p	< 0.01	> 0.05	< 0.001	< 0.02	

TABLE 10.8

NORMAL LIMITS FOR PLASMA FREE CHOLESTEROL  
AND CHOLESTEROL ESTER LEVELS

limits (mg/100 ml) calculated from normal limits  
for total cholesterol (Table 10.1) and taking  
free cholesterol:cholesterol ester as 1:2

Age (years)	free cholesterol	cholesterol ester
0 - 19	40 - 77	80 - 154
20 - 29	40 - 80	80 - 160
30 - 39	47 - 90	94 - 180
40 - 49	50 - 103	100 - 206
50 - 59	53 - 110	106 - 220

TABLE 10.9

ABSOLUTE LEVELS OF PLASMA FREE AND ESTER CHOLESTEROL  
IN GROUPS OF SUBJECTS WITH OBSTRUCTIVE JAUNDICE

results (mean and standard deviation) as mg/100 ml

No. of subjects	Group I (10)	Group II (2)	Group III (8)	I + II + III (20)	Controls (IV) (5)
<u>Free cholesterol</u>	144 ± 66	120 ± 14	347 ± 171	223 ± 275	52 ± 7
<u>Ester cholesterol</u>	205 ± 93	272 ± 59	103 ± 35	171 ± 198	122 ± 17

Significances

free cholesterol

	I - II	I - III	II - III
t	0.4958	3.4800	1.7800
p	> 0.05	< 0.005	> 0.05

	I - IV	II - IV	III - IV	(I + II + III) - IV
t	3.2934	4.1430	3.7974	2.4377
p	< 0.01	< 0.01	< 0.005	< 0.025

ester cholesterol

TABLE 10.9 (contd.)

		<u>ester cholesterol</u>			
		I - II	I - III	II - III	(I + II + III)-IV
t		0.9550	2.9213	5.5244	
p		> 0.05	< <u>0.01</u>	< <u>0.001</u>	
t		1.9373	5.8969	1.1377	1.1663
p		> 0.05	< <u>0.005</u>	> 0.05	> 0.05

TABLE 10.10

PLASMA LIPOPROTEIN CHOLESTEROL LEVELS OF INDIVIDUAL SUBJECTS  
WITH OBSTRUCTIVE JAUNDICE

results as mg/100 ml

	Subject no.	VLDL	LDL	HDL
Group I	1	26	317	27
	2	56	309	11
	3	16	257	31
	4	58	209	21
	5	3	147	33
	6	27	183	69
	7	8	136	30
	8	38	257	51
	9	3	563	102
	10	36	234	43

Group II/

TABLE 10.10 (contd.)

Group II	11	32	237	91
	12	12	324	87
Group III	13	16	203	13
	14 (Dec. 14th)	60	568	12
	15	204	579	17
	16 (Mar. 7th)	160	345	21
	17	44	365	33
	18	"	"	"
	19	47	329	7
	20	22	214	24
	21	36	234	43

Controls (IV)/

TABLE 10.10 (contd.)

Controls (IV)	T	55	106	30
	M	15	91	48
	B	18	116	22
	L	16	117	59
	E	30	85	33

TABLE 10.11

PLASMA LIPOPROTEIN CHOLESTEROL LEVELS IN GROUPS OF SUBJECTS  
WITH OBSTRUCTIVE JAUNDICE

results (mean and standard deviation) as mg/100 ml

Group	No.	VLDL	LDL	HDL
I	10	27 ± 20	261 ± 122	42 ± 27
II	2	22 ± 14	281 ± 62	89 ± 3
III	8	74 ± 69	354 ± 149	21 ± 12
I + II + III	20	45 ± 50	300 ± 132	38 ± 28
IV (Controls)	5	27 ± 17	103 ± 15	38 ± 15

Significances/

TABLE 10.11 (contd.)

Significances	t	p	t	p	t	p	t	p
I - II			0.3360	> 0.05	0.2116	> 0.05	2.4102	< <u>0.05</u>
I - III			2.0322	> 0.05	1.4658	> 0.05	2.0176	> 0.05
II - III			1.0042	> 0.05	0.6675	> 0.05	7.6593	< <u>0.001</u>
I - IV			0.0286	> 0.05	2.8275	< <u>0.02</u>	0.2625	> 0.05
II - IV			0.3506	> 0.05	6.9744	< <u>0.001</u>	4.1780	< <u>0.01</u>
III - IV			1.4609	> 0.05	3.8790	< <u>0.005</u>	2.3019	< <u>0.05</u>
(I + II + III)-IV			0.7938	> 0.05	3.5159	< <u>0.005</u>	0.0076	> 0.05

TABLE 10.12

LIPOPROTEIN COMPOSITION IN SUBJECTS DURING AND AFTER JAUNDICE

<u>Subject no. 14</u>	Dec. 27th	Feb. 2nd
jaundice	+	-
<u>LDL % composition</u>		
cholesterol	32	30
triglyceride	8	17
phospholipid	44	32
protein	15	21
<u>HDL lipid (mg/100 ml)</u>		
cholesterol	34	12
phospholipid	124	50
phospholipid:cholesterol	3.64	4.16

Subject no. 16/

TABLE 10.12 (contd.)

<u>Subject no. 16</u>	Mar. 7th	Mar. 11th	Mar. 20th	Mar. 29th
jaundice	+	+	+	-
<u>LDL % composition</u>				
cholesterol	21	23	25	32
triglyceride	20	23	15	9
phospholipid	41	42	44	29
protein	17	12	16	30
<u>HDL lipid (mg/100 ml)</u>				
cholesterol	14	14	12	37
phospholipid	63	64	28	79
phospholipid:cholesterol	4.50	4.57	2.33	2.13

TABLE 10.13

CONCENTRATIONS OF CHOLESTEROL AND PHOSPHOLIPID  
 FROM d > 1.063 FRACTION OF INDIVIDUAL SUBJECTS WITH OBSTRUCTIVE JAUNDICE

cholesterol and phospholipid concentrations in mg/100 ml

	Subject no.	Cholesterol	Phospholipid	$\frac{\text{Phospholipid}}{\text{Cholesterol}}$
Group I	1	40	134	3.55
	2	18	76	4.21
	3	42	117	2.79
	4	31	102	3.29
	5	40	204	5.10
	6	71	202	2.84
	7	20	71	3.54
	8	51	74	2.64
	9	133	-	-
Group II/	10	33	106	3.21

TABLE 10.13 (contd.)

Group II	11	81	230	2.84
	12	88	196	2.45
Group III	13	19	76	4.00
	14 (Dec. 27th)	34	124	3.64
	15	9	44	4.92
	16 (Mar. 7th)	14	63	4.50
	17	16	67	4.18
	18	-	-	-
	19	15	38	2.53
	20	20	60	3.00
Controls (IV)	21	22	84	3.82

TABLE 10.13 (contd.)

Controls (IV)	T	39	90	2.31
	M	44	76	1.73
	B	26	42	1.62
	L	44	93	2.11
	E	25	46	1.84

TABLE 10.14

CONCENTRATIONS OF CHOLESTEROL AND PHOSPHOLIPID AND PHOSPHOLIPID:CHOLESTEROL RATIOS FROM  $d > 1.063$  FRACTION OF GROUPS OF SUBJECTS WITH OBSTRUCTIVE JAUNDICE  
cholesterol and phospholipid concentrations in mg/100 ml

Group	Cholesterol	Phospholipid	$\frac{\text{Phospholipid}}{\text{Cholesterol}}$
I	48 $\pm$ 34 (10)	122 $\pm$ 48 (9)	3.30 $\pm$ 0.86 (9)
II	85 $\pm$ 5 (2)	213 $\pm$ 24 (2)	2.65 $\pm$ 0.28 (2)
III	19 $\pm$ 7 (8)	70 $\pm$ 27 (8)	3.82 $\pm$ 0.77 (8)
I + II + III	40 $\pm$ 31 (20)	110 $\pm$ 57 (19)	3.44 $\pm$ 0.85 (19)
IV (Controls)	36 $\pm$ 9 (5)	69 $\pm$ 24 (5)	1.92 $\pm$ 0.28 (5)

Significances/

TABLE 10.14 (contd.)

Significances	t	p	t	p	t	p	t	p
I - II	1.4832	> 0.05	2.4148	< <u>0.05</u>	1.4130	> 0.05		
I - III	2.4082	< <u>0.05</u>	2.5313	< <u>0.025</u>	0.9540	> 0.05		
II - III	11.6626	< <u>0.001</u>	6.8717	< <u>0.001</u>	2.0387	> 0.05		
I - IV	0.7908	> 0.05	2.0869	> 0.05	4.2042	< <u>0.005</u>		
II - IV	6.6893	< <u>0.005</u>	7.1253	< <u>0.001</u>	3.0678	< <u>0.05</u>		
III - IV	3.6277	< <u>0.005</u>	0.0068	> 0.05	5.2018	< <u>0.001</u>		
(I + II + III)-IV	0.2947	> 0.05	1.9239	> 0.05	4.3728	< <u>0.001</u>		
		> 0.05	> 0.05	> 0.05	< <u>0.001</u>	< <u>0.001</u>		

TABLE 10.15

RELATIVE LIPID AND PROTEIN COMPOSITION OF LOW DENSITY LIPOPROTEIN  
 FRACTION OF INDIVIDUAL SUBJECTS WITH OBSTRUCTIVE JAUNDICE

results expressed as a percentage of the total low density

		Lipoprotein concentration			
Group	Subject no.	Cholesterol	Triglyceride	Phospholipid	Protein
Group I	1	35	9	35	21
	2	24	22	27	24
	3	33	13	28	26
	4	31	16	29	24
	5	29	12	45	14
	6	36	6	36	22
Group II/	7	32	18	30	21
	8	41	6	27	26
	9	-	-	-	-
	10	31	11	38	20

TABLE 10.15 (contd.)

Group II	11	32	5	35	28
	12	38	4	33	25
Group III	13	36	13	34	17
	14 (Dec. 27th)	32	8	44	15
	15	30	8	50	13
	16 (Mar. 7th)	21	20	41	17
	17	31	11	43	16
	18	-	-	-	-
	19	30	9	46	15
	20	30	5	47	18
	21	32	9	49	10

Controls (IV)/

TABLE 10.15 (contd.)

Controls (IV)	T	40	7	26	27
	M	34	8	24	33
	B	35	6	27	32
	L	38	13	28	21
	E	37	8	24	32

TABLE 10.16

RELATIVE LIPID AND PROTEIN COMPOSITION OF LOW DENSITY LIPOPROTEIN  
 FRACTION OF GROUPS OF SUBJECTS WITH OBSTRUCTIVE JAUNDICE AND CONTROLS

results (mean and standard deviation) expressed as a percentage of  
 the total lipoprotein concentration

Group	No.	Cholesterol	Triglyceride	Phospholipid	Protein
I	9	32.4 ± 4.8	12.6 ± 5.4	32.8 ± 6.2	22.0 ± 3.7
II	2	35.0 ± 4.2	4.5 ± 0.7	34.0 ± 1.4	26.5 ± 2.1
III	8	30.3 ± 4.2	10.6 ± 4.5	43.6 ± 4.8	15.9 ± 1.6
I + II + III	19	31.8 ± 4.5	10.8 ± 5.2	37.7 ± 7.7	19.1 ± 6.1
IV (Controls)	5	36.8 ± 2.4	8.4 ± 2.7	25.8 ± 1.8	29.0 ± 5.1

Significances/

TABLE 10.16 (contd.)

Significances							
I - II	t	0.6966	2.0265	0.2683	1.6139		
	p	> 0.05	> 0.05	> 0.05	> 0.05		
I - III	t	1.0030	0.8962	4.1433	4.3751		
	p	> 0.05	> 0.05	< 0.001	< 0.001		
II - III	t	1.4186	1.7493	2.6925	5.6778		
	p	> 0.05	> 0.05	< 0.05	< 0.001		
I - IV	t	1.8985	1.5962	2.4365	2.9858		
	p	> 0.05	> 0.05	< 0.05	< 0.02		
II - IV	t	0.7531	1.9126	5.6966	0.6474		
	p	> 0.05	> 0.05	< 0.005	> 0.05		
III - IV	t	3.1291	0.8734	7.6611	6.6157		
	p	< 0.001	> 0.05	< 0.001	< 0.001		
(I + II + III)-IV	t	2.3678	0.9832	3.3732	3.6803		
	p	< 0.05	> 0.05	< 0.005	< 0.005		

**FIGURES**

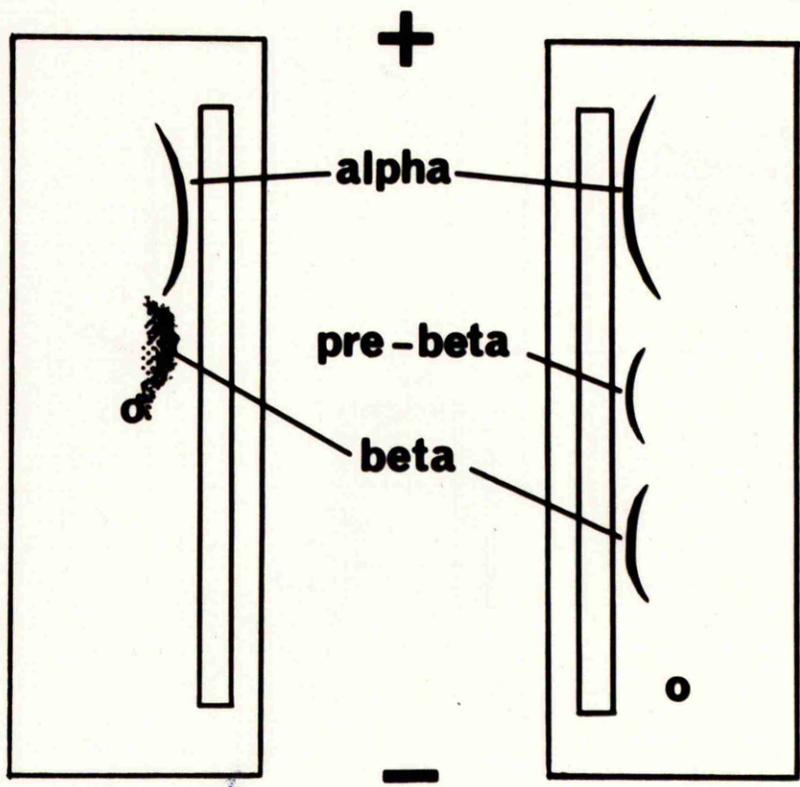
FIGURES  
CHAPTER 4

Legend for Fig. 4.1

DIAGRAMMATIC REPRESENTATION OF  
ELECTROPHORETIC MOBILITIES OF PLASMA  
LIPOPROTEINS IN AGAR AND AGAROSE GELS

It should be noted that

- (a) beta lipoprotein hardly migrates in agar and tends to react with the gel.
- (b) pre-beta lipoprotein is not distinguishable from beta lipoprotein in agar but has an independent mobility in agarose.



**2% AGAR.**

**1% AGAROSE.**

Legend for Fig. 4.2

IMMUNOELECTROPHORESIS OF NORMAL PLASMA  
IN AGAR GEL

The antiserum in the left-hand trough was R5 with anti-alpha and anti-beta lipoprotein reactivity.

The antiserum in the right-hand trough was Hyland anti-beta lipoprotein antiserum.

The appearances, which are typical of normal plasma, produced with R5 are of a beta lipoprotein precipitin arc which has hardly migrated from the origin, and a faster moving alpha lipoprotein precipitin arc. The mono-specific anti-beta lipoprotein antiserum has reacted with the beta lipoprotein only. Some beta lipoprotein has typically interacted with the gel. Very low density lipoprotein is not distinguished from beta lipoprotein with this system.

In/

In this slide and in all the other immuno-electrophoretic investigations which are subsequently described, all precipitin lines which are held to be due to lipoprotein were shown to stain for lipid with Oil red O.



Legend for Fig. 4.3

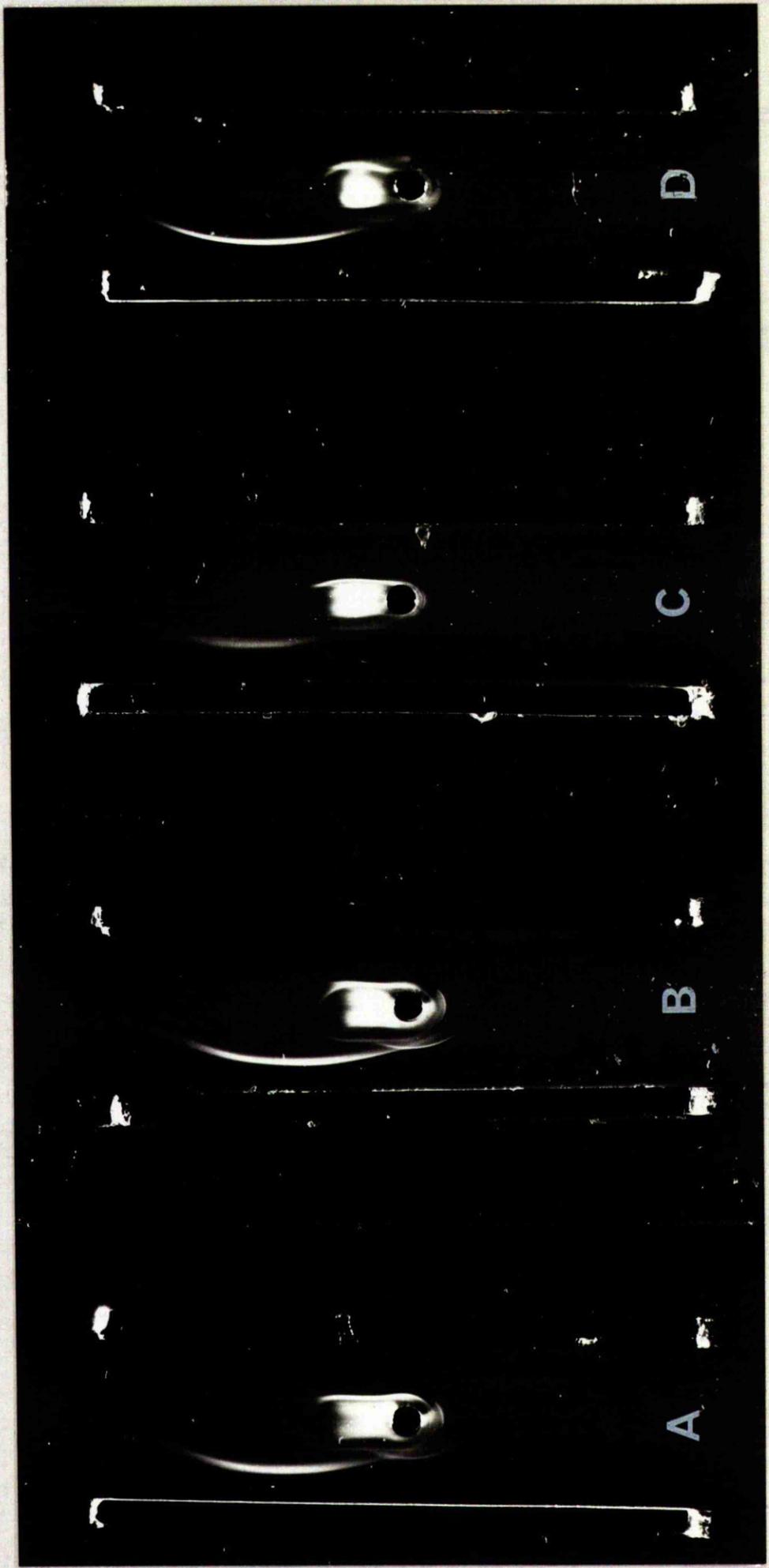
IMMUNOELECTROPHORESIS OF PLASMA IN AGAR GEL  
SHOWING ADDITIONAL PRECIPITIN LINE

Plasma samples from subject no. 20.

The left-hand trough in each slide was loaded with antiserum R5 with anti-alpha and anti-beta lipoprotein reactivity. Each right-hand trough contained Hyland anti-beta lipoprotein antiserum.

In A and B (samples of May 13th) the plasma has reacted with R5 to produce normal beta and alpha lipoprotein precipitin arcs, and a 3rd arc which has migrated slightly towards the cathode. The additional arc has not been seen, by R5, in slides C (sample of May 29th) or D (sample of June 10th).

The additional arc has also not been produced by the monospecific anti-beta lipoprotein antiserum.



A

B

C

D





Legend for Fig. 4.4b

IMMUNOELECTROPHORESIS OF PLASMA IN AGAR GEL  
SHOWING PRODUCTION OF ADDITIONAL PRECIPITIN  
ARC BY SEVERAL ANTI-ALPHA LIPOPROTEIN ANTISERA

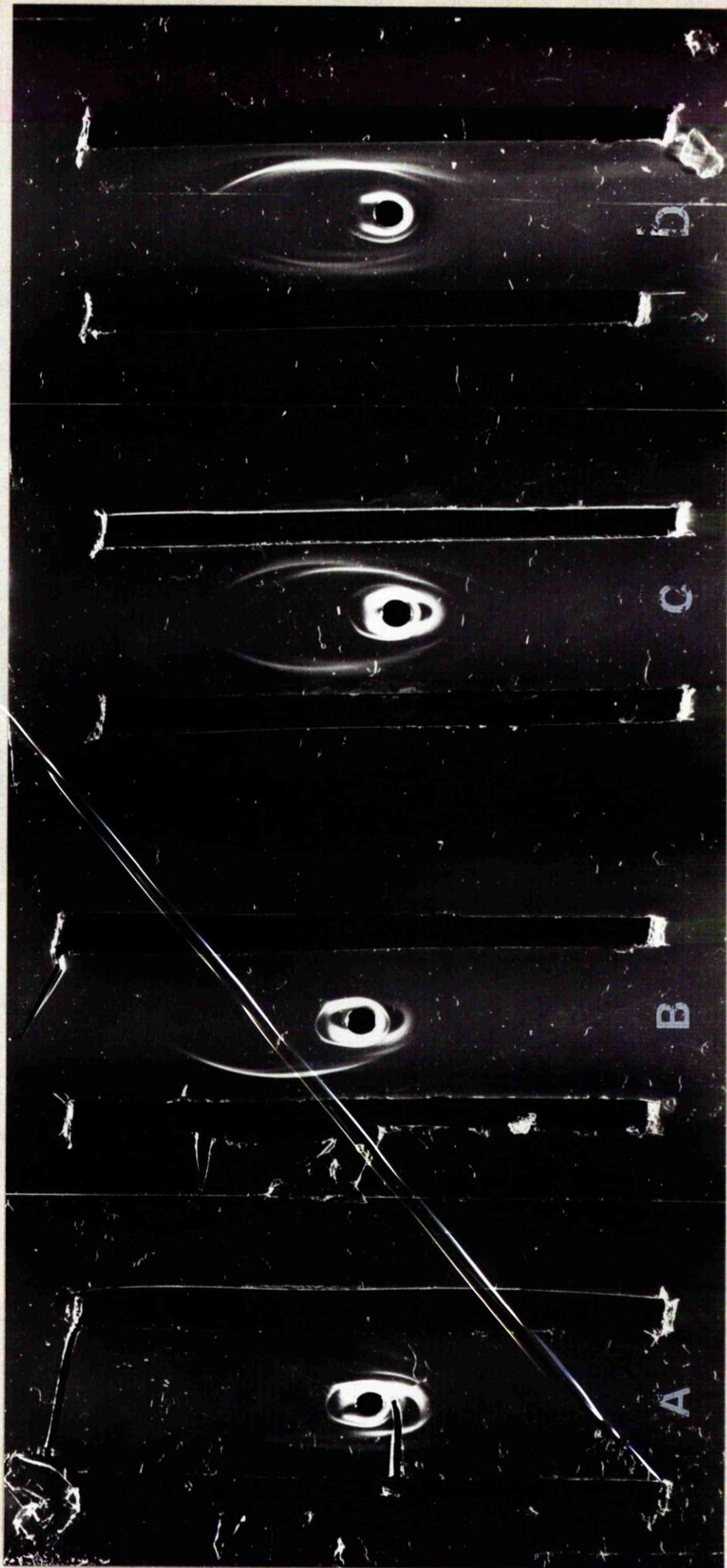
Plasma of subject no. 14 (Dec. 27th).

The troughs were loaded with the following antisera reading from left to right

A	R1, 51	B	124A, 360
C	alpha LpT <sub>1</sub> , R2	D	RGA, ZAZ 2

Antisera R1, 51, 124A, alpha LpT<sub>1</sub> and R2 have each reacted with the plasma to produce the new cathodal-migrating precipitin arc. It has not been produced with the monospecific anti-beta lipoprotein antiserum 360.

A dense material which has reacted with the gel is seen internal to the new precipitin arc.



Legend for Fig. 4.5

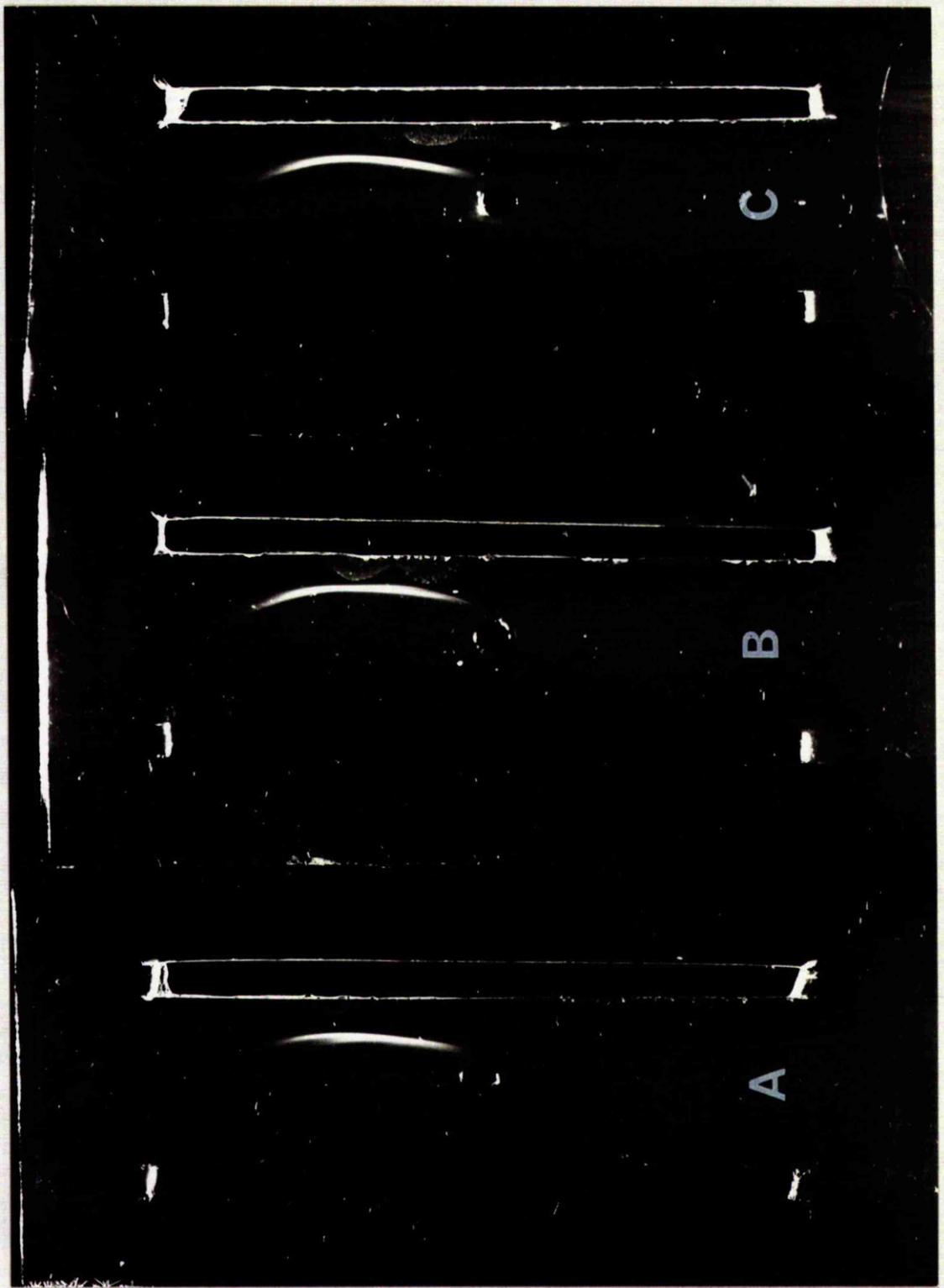
IMMUNOELECTROPHORESIS OF HIGH DENSITY  
LIPOPROTEIN FRACTION IN AGAR GEL FROM  
SUBJECT WITH ADDITIONAL PRECIPITIN ARC  
IN LOW DENSITY LIPOPROTEIN FRACTION  
SHOWING NORMAL ALPHA LIPOPROTEIN  
PRECIPITIN ARC ONLY

A Fraction of subject no. 14 (Dec.  
27th).

The antiserum in each left-hand trough was monospecific Hyland anti-beta lipoprotein antiserum and in each right-hand trough R5 with anti-alpha and anti-beta lipoprotein reactivity. Normal alpha lipoprotein precipitin arcs have been produced with R5 but there is no evidence of the additional precipitin arc seen in the corresponding low density lipoprotein fraction.

In B and C the high density fraction of subjects/

subjects nos. 1 and 4 respectively, with no abnormal precipitin arc in their low density fraction, was run. It yielded, with R5, a normal alpha lipoprotein precipitin arc in both cases.



Legend for Fig. 4.6

IMMUNOELECTROPHORESIS OF  $d > 1.006$   
LIPOPROTEIN FRACTION IN AGAR GEL SHOWING  
ADDITIONAL PRECIPITIN ARC

Fraction of A subject no. 14 (Dec. 14th)

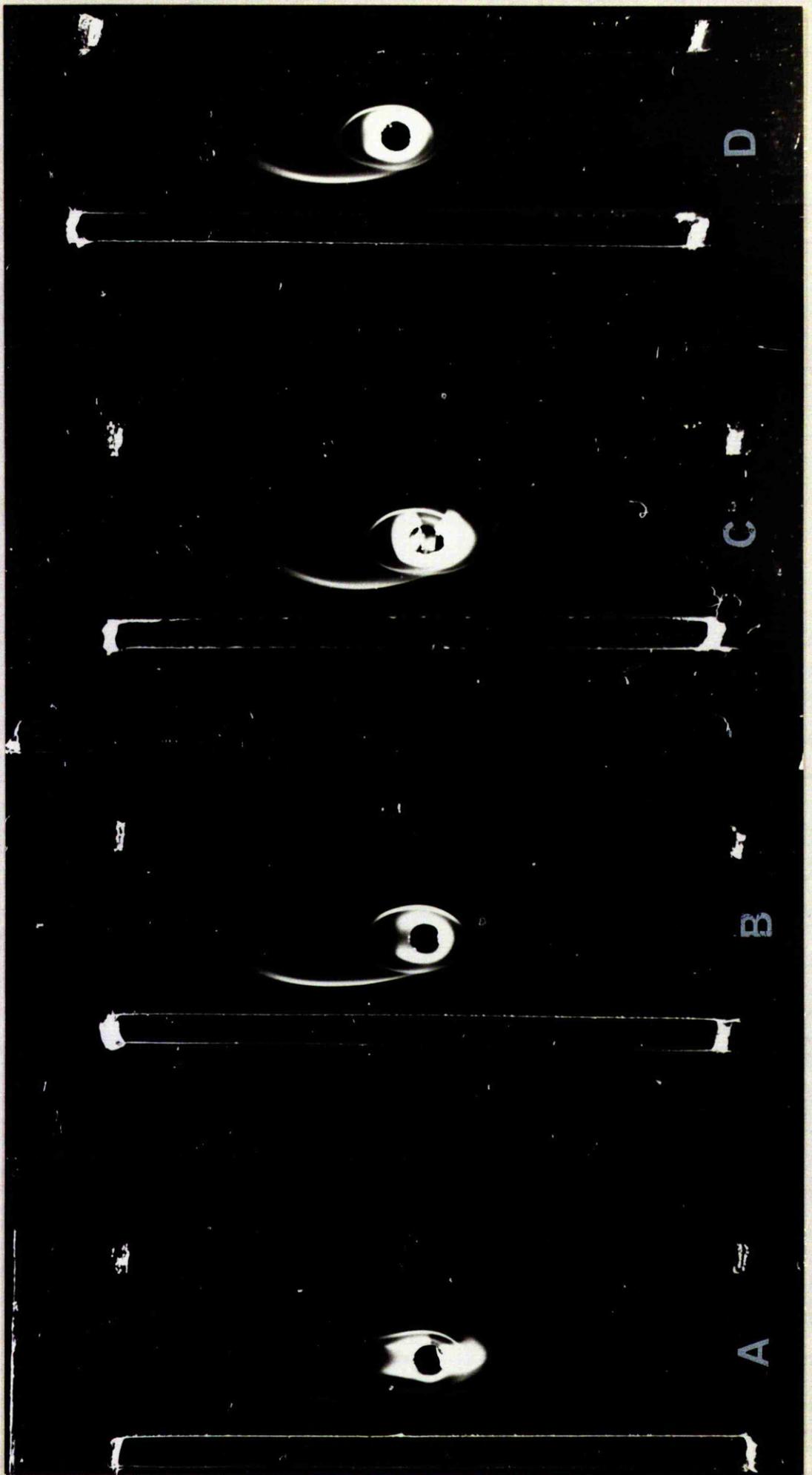
B subject no. 14 (Feb. 2nd)

C subject no. 15

D subject no. 17

The antiserum in each left-hand trough was R5 and in each right-hand trough Hyland anti-beta. In A, C and D, in addition to the normal alpha lipoprotein and beta lipoprotein precipitin arcs, R5 has produced a cathodal-migrating arc. The monospecific Hyland anti-beta lipoprotein antiserum has resulted, for each sample, in a beta lipoprotein precipitin arc only. In B the fraction obtained following the relief of jaundice and the fall in plasma/

plasma lipids in subject no. 14 no longer shows  
the additional arc; instead the normal alpha  
lipoprotein precipitin arc which is faint in  
his earlier sample in A is more prominent.

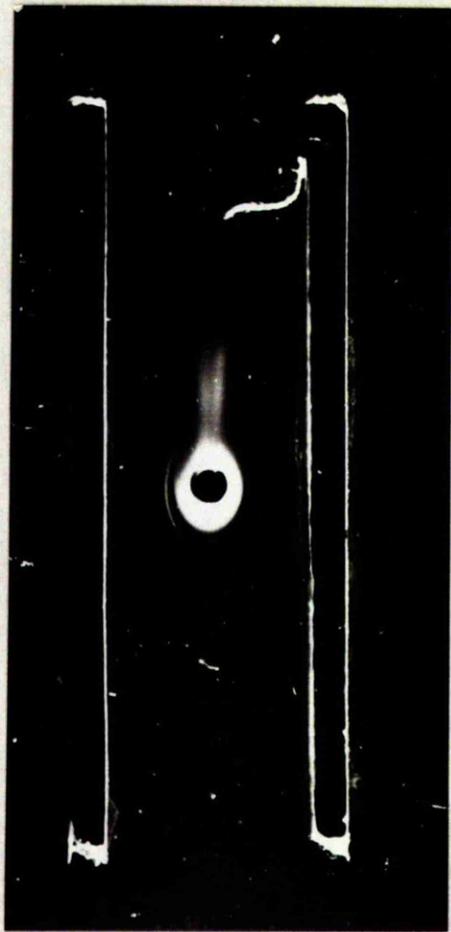


Legend for Fig. 4.7

IMMUNOELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION IN AGAR GEL  
SHOWING ADDITIONAL PRECIPITIN ARC

Fraction of subject no. 15.

The left-hand trough contained antisera R5 with anti-alpha and anti-beta lipoprotein reactivity; a sharp precipitin arc which has barely migrated cathodally has been produced. The right-hand trough contained antiserum 360 with anti-beta lipoprotein reactivity only and no cathodal-migrating precipitin arc corresponding to that produced with R5 is found. Both antisera have produced indistinct beta lipoprotein precipitin arcs.



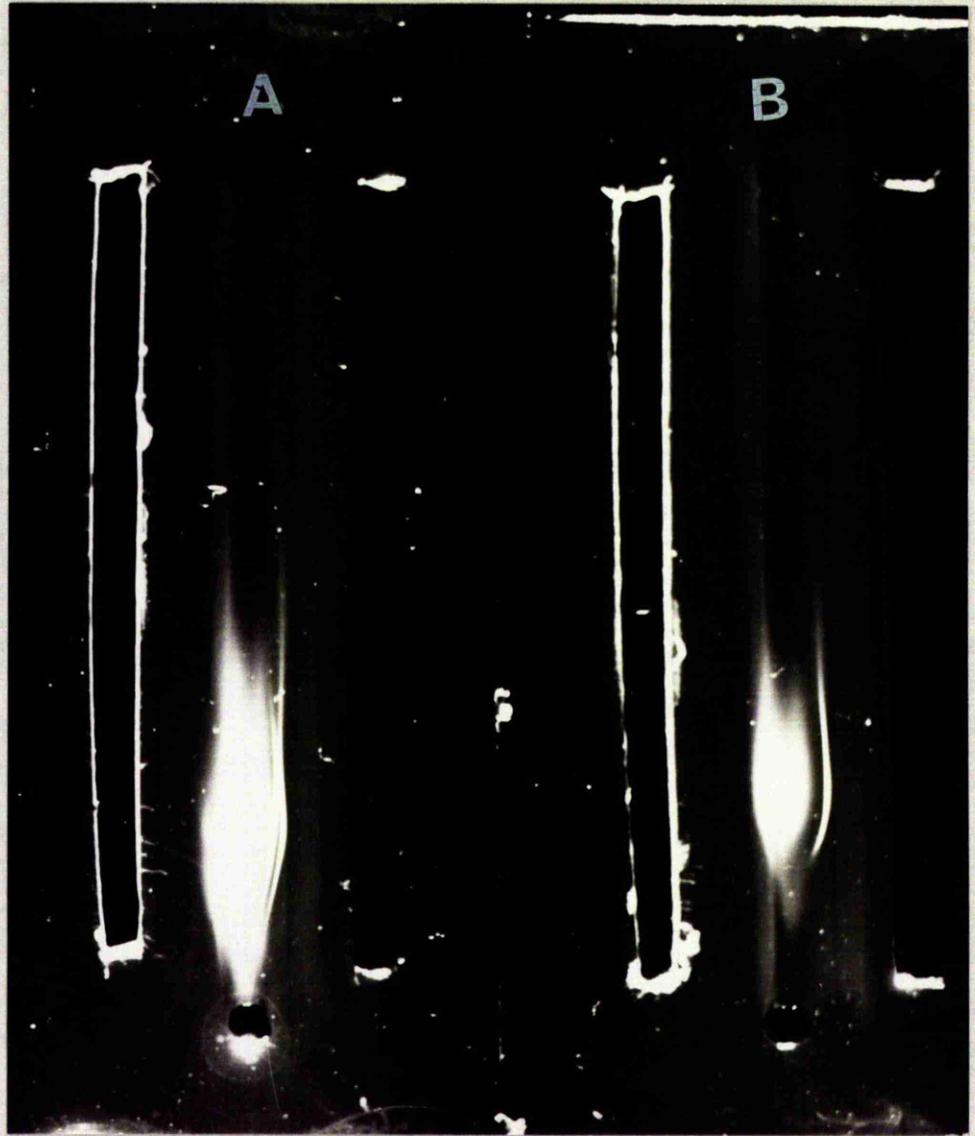
Legend for Fig. 4.8

IMMUNOELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION IN AGAROSE GEL  
SHOWING ADDITIONAL PRECIPITIN ARC

Fraction of A subject no. 15

B subject no. 17

The antiserum in each right-hand trough was the polyvalent antiserum AHS. In B only a normal beta lipoprotein precipitin arc has resulted; in A an additional arc is also visible, displaying an electrophoretic mobility close to that of the beta lipoprotein. The ill-defined precipitate running through the centre of each slide can be ascribed to the polyvalent antiserum AHS having reacted with components of the antiserum, ZAZ 2, which had been added to the left-hand trough.

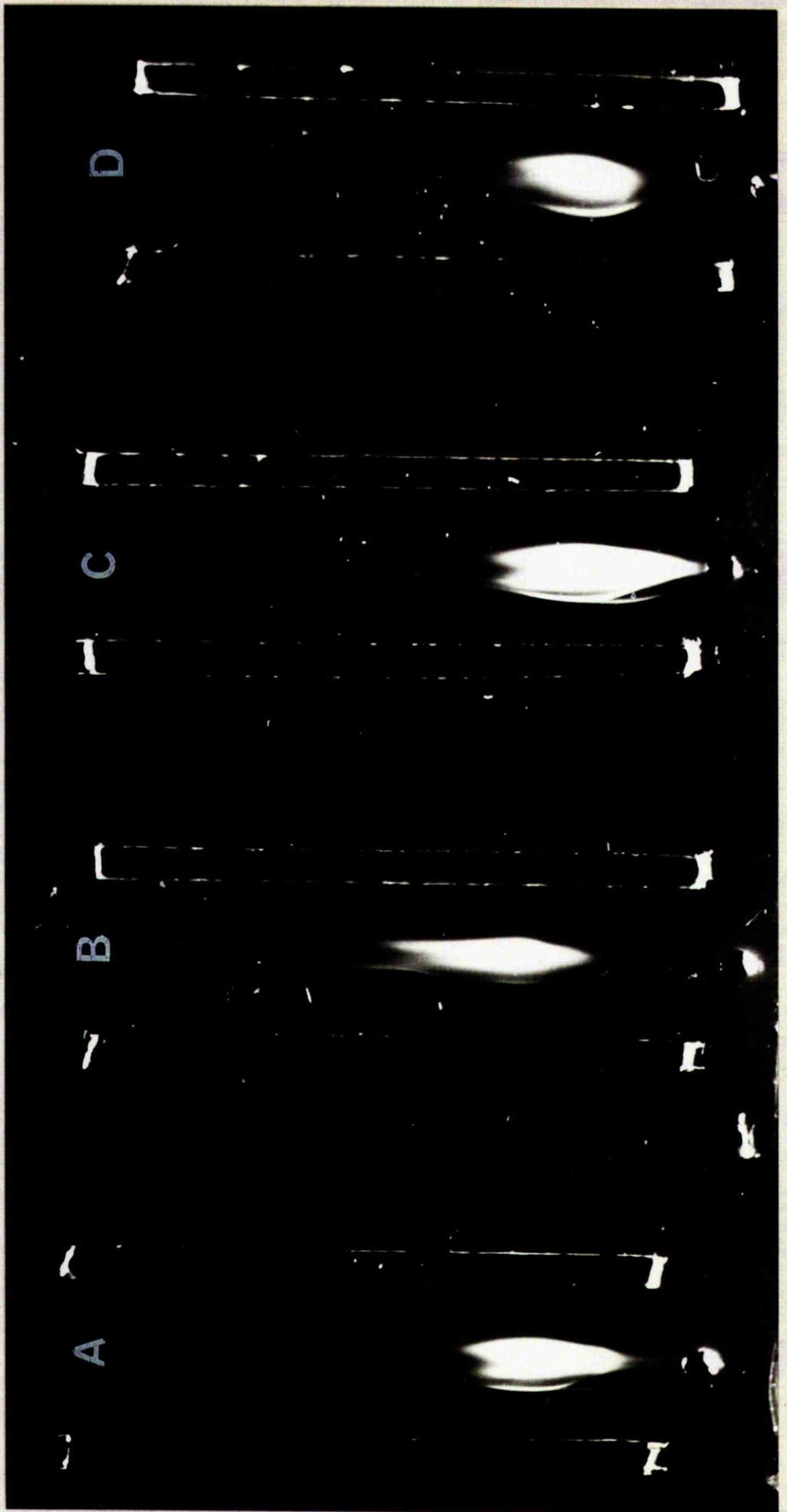


Legend for Fig. 4.9

IMMUNOELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION IN AGAROSE GEL  
SHOWING ADDITIONAL PRECIPITIN ARC

Fractions of A subject no. 14 (Dec. 14th)  
B subject no. 14 (Feb. 2nd)  
C subject no. 15  
D subject no. 17

The antiserum in each left-hand trough was 79 with anti-alpha and anti-beta lipoprotein reactivity. In A and C in addition to the beta lipoprotein precipitin arc produced by the antiserum, a portion of an additional arc is present. The additional arc in the LDL fraction in A is no longer evident in the same subject's low density lipoprotein fraction in B obtained when the jaundice had been relieved. A beta lipoprotein precipitin arc only is seen in D though an additional arc was seen in agar.



Legend for Fig. 4.10

IMMUNODIFFUSION OF LOW DENSITY LIPOPROTEIN  
FRACTION IN OUCHTERLONY PLATE SHOWING  
ADDITIONAL PRECIPITIN LINE

The centre wells of A and B contained anti-serum 124A with anti-alpha and anti-beta lipoprotein reactivity.

The centre wells of C and D contained the monospecific anti-beta lipoprotein antiserum 360.

The peripheral wells were loaded with low density lipoprotein fraction of

1. subject no. 14 (Dec. 14th)
2. subject no. 14 (Feb. 2nd)
3. subject no. 15

The top and bottom wells in each series contained low density lipoprotein from a normal subject.

The/

The remaining wells contained low density lipoprotein from other subjects in the series.

The fraction in 1 and 3 has reacted with antiserum 124A to produce 2 precipitin lines; the inner line shows a reaction of identity with the lines derived from the low density lipoprotein of both the normal and the other jaundiced subjects. The additional line in 1 and 3 has not been produced by antiserum 360, which has resulted in a single line giving a reaction of identity with that of normal beta lipoprotein. In 2 the fraction derived from subject no. 14 following relief of jaundice, no longer shows the additional line with antiserum 124A.

1 2



C

3

2 1



B

1 2



A

3

2 1



D

Legend for Fig. 4.11

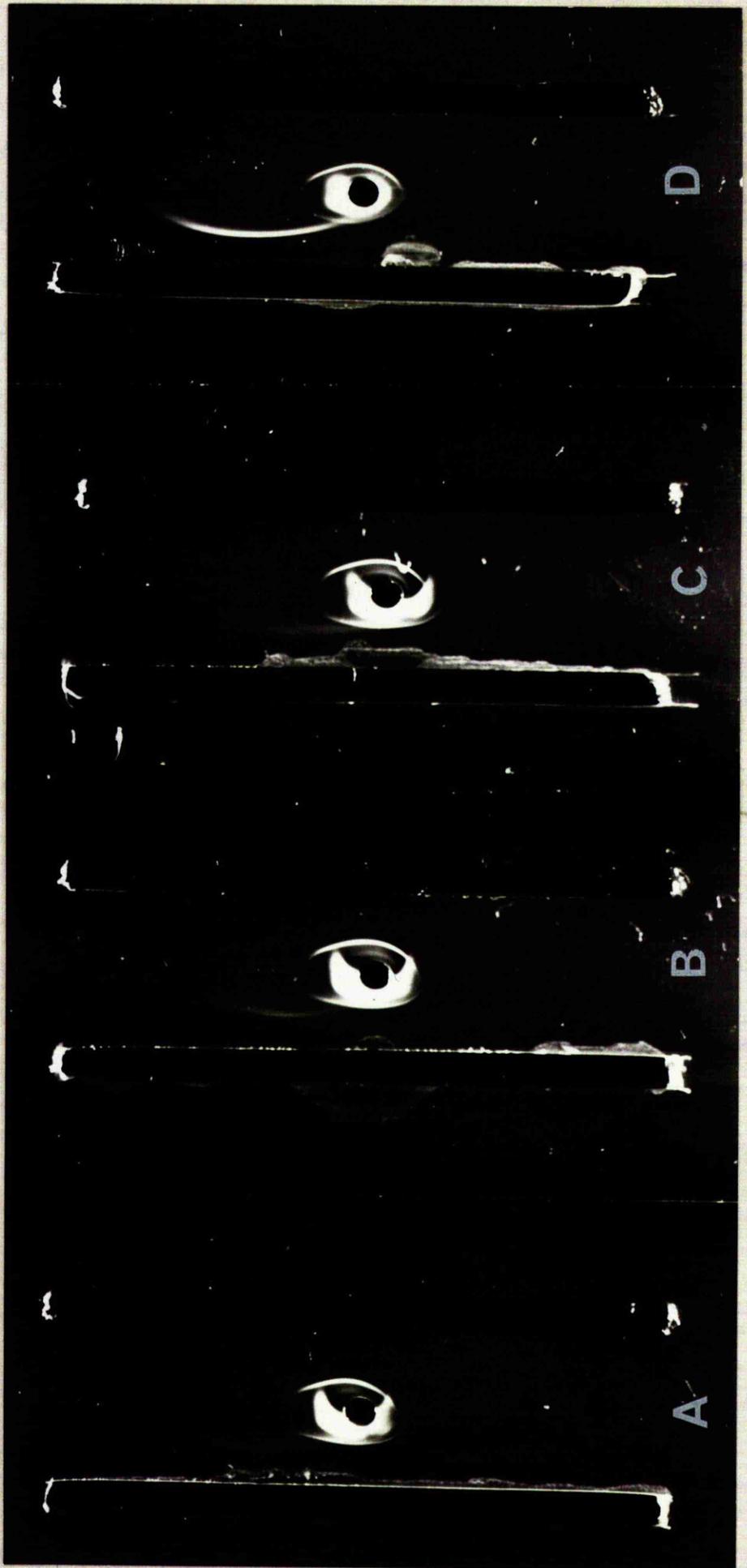
IMMUNOELECTROPHORESIS OF PLASMA IN AGAR  
GEL SHOWING DISAPPEARANCE OF ADDITIONAL  
PRECIPITIN ARC FOLLOWING RELIEF OF  
OBSTRUCTIVE JAUNDICE

Plasma of subject no. 16

A Mar. 7th                      B Mar. 11th  
C Mar. 20th                     D Mar. 29th

The antiserum in each left-hand trough was R5 with anti-alpha and anti-beta lipoprotein reactivity, and in each right-hand trough was Hyland anti-beta lipoprotein. In A, B and C the posterior spur of the additional cathodal-migrating precipitin arc has been produced with R5, together with dense material which has reacted with the gel. Faint normal alpha lipoprotein precipitin arcs are also present. The plasma in D taken when the jaundice had been relieved and the plasma lipids/

lipids had fallen considerably, no longer shows the additional arc or the gel-reacting material with antiserum R5, but shows the reappearance of a normal alpha lipoprotein arc. The monospecific Hyland anti-beta lipoprotein antiserum has produced, in all cases, a single beta lipoprotein precipitin arc. See Table 10.4 for corresponding plasma lipid levels.



Legend for Fig. 4.12

IMMUNODIFFUSION OF LOW DENSITY LIPOPROTEIN  
FRACTION IN OUCHTERLONY PLATE SHOWING  
DISAPPEARANCE OF ADDITIONAL PRECIPITIN LINE  
FOLLOWING RELIEF OF OBSTRUCTIVE JAUNDICE

The centre wells contained the following  
antisera reading from left to right and top to  
bottom

124A, 360, ZAZ 2, Hyland anti-beta,  
RGA, R1, R5, alpha L<sub>p</sub>T<sub>1</sub>, alpha L<sub>p</sub>T<sub>2</sub>,  
AHS, 79, 81.

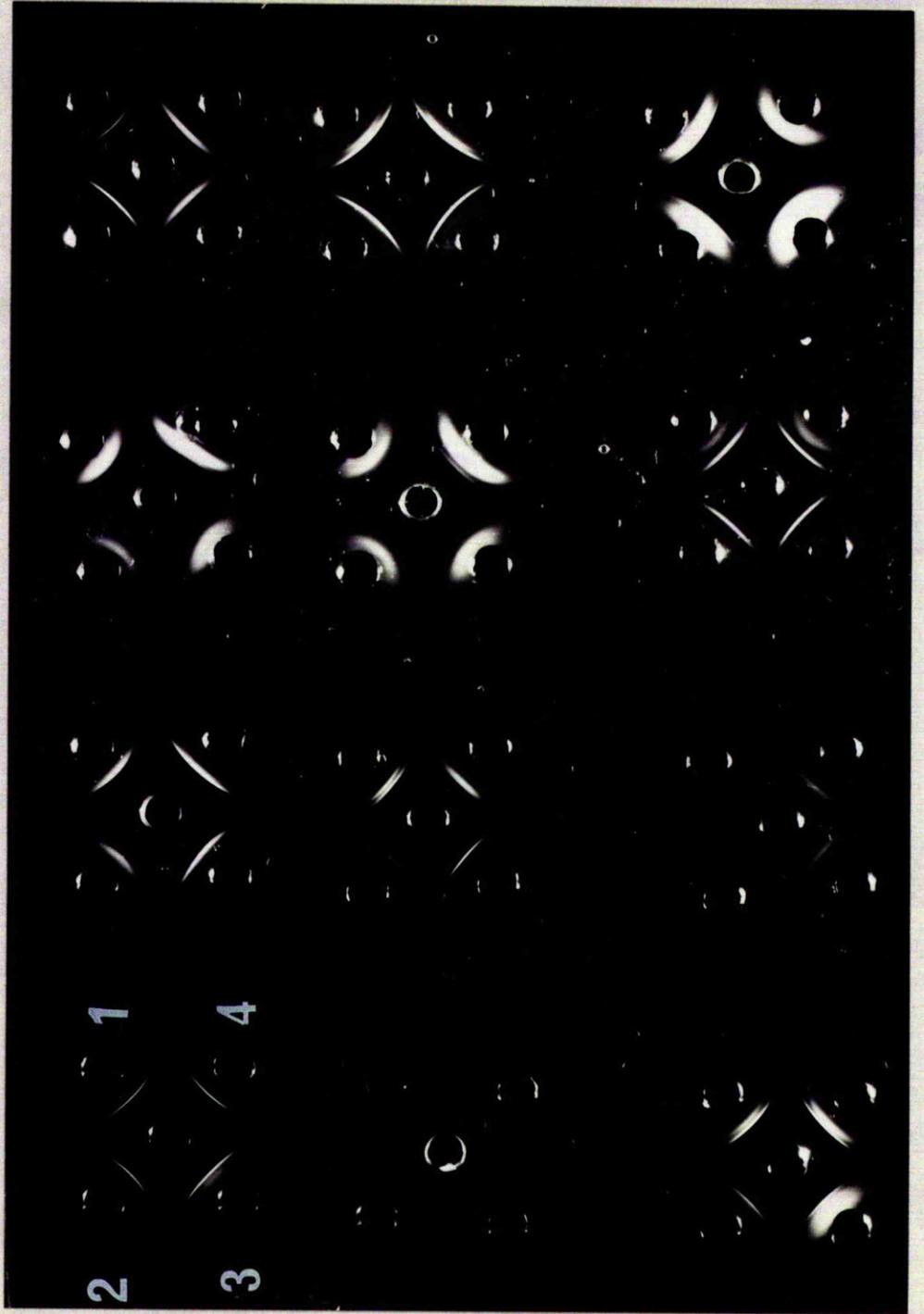
The first set of peripheral wells was loaded  
with the low density lipoprotein fraction of

1. subject no. 14 (Dec. 14th)
2. subject no. 14 (Feb. 2nd)
3. a normal subject
4. subject no. 15

The other sets of wells were similarly loaded.

In 1 and 4, 2 distinct precipitin lines  
have/

have been produced by several of the antisera, all of which have anti-alpha and anti-beta lipoprotein reactivity, but only a single line has been produced by the monospecific anti-beta lipoprotein antisera 360 and Hyland anti-beta. In 2 and 3 a single beta lipoprotein precipitin line has been produced by each of the antisera.



Legend for Fig. 4.13

IMMUNODIFFUSION OF LOW DENSITY LIPOPROTEIN  
FRACTION IN OUCHTERLONY PLATE SHOWING  
DISAPPEARANCE OF ADDITIONAL PRECIPITIN LINE  
FOLLOWING REACTION WITH ANTISERUM WITH  
ANTI-ALPHA AND ANTI-BETA LIPOPROTEIN  
REACTIVITY, BUT NOT WITH MONOSPECIFIC  
ANTI-BETA LIPOPROTEIN ANTISERUM

Fraction of subject no. 14 (Dec. 14th).

The centre well contained antiserum R5  
with anti-alpha and anti-beta lipoprotein  
reactivity.

The peripheral wells were loaded as  
follows

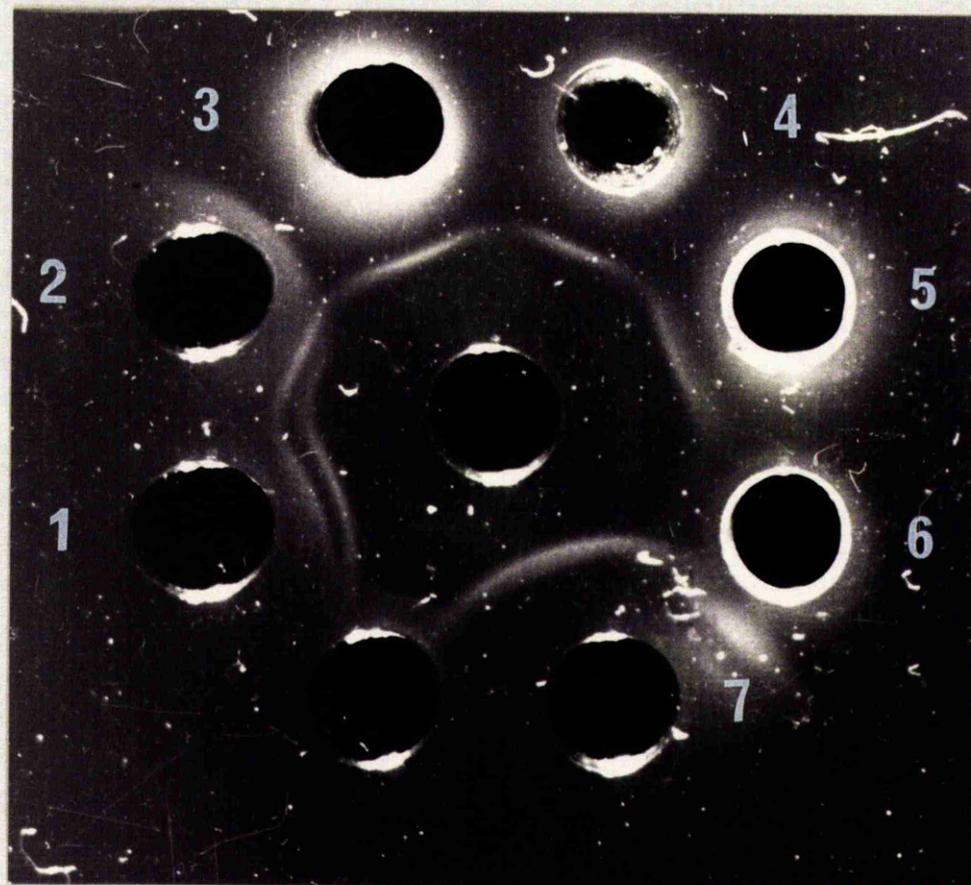
- 1 and 2    supernate from 0.1 ml low density  
          fraction diluted with saline two-  
          and three-fold respectively.
- 3 and 4    supernate from 0.1 ml low density  
          fraction previously reacted with  
          0.2 ml/

0.2 ml and 0.3 ml antiserum 360  
respectively (monospecific anti-  
beta lipoprotein antiserum).

5 and 6 supernate from 0.1 ml low density  
fraction previously reacted with  
0.2 ml and 0.3 ml respectively of  
antiserum 124A (anti-alpha and  
anti-beta lipoprotein antiserum).

7 low density fraction from normal  
subject.

Both saline-diluted fractions show 2  
precipitin lines (1, 2). The 2 fractions  
previously reacted with the anti-beta lipo-  
protein antiserum 360 show removal of the  
internal precipitin line only (3, 4). The  
fraction previously absorbed with the larger  
volume of antiserum 124A shows removal of  
both precipitin lines (6).



Legend for Fig. 4.14

IMMUNODIFFUSION IN OUCHTERLONY PLATE SHOWING  
REMOVAL OF ANTI-ALPHA LIPOPROTEIN REACTIVITY  
FROM POLYVALENT ANTISERUM FOLLOWING ITS  
REACTION WITH THE LOW DENSITY LIPOPROTEIN  
FRACTION OF A JAUNDICED SUBJECT

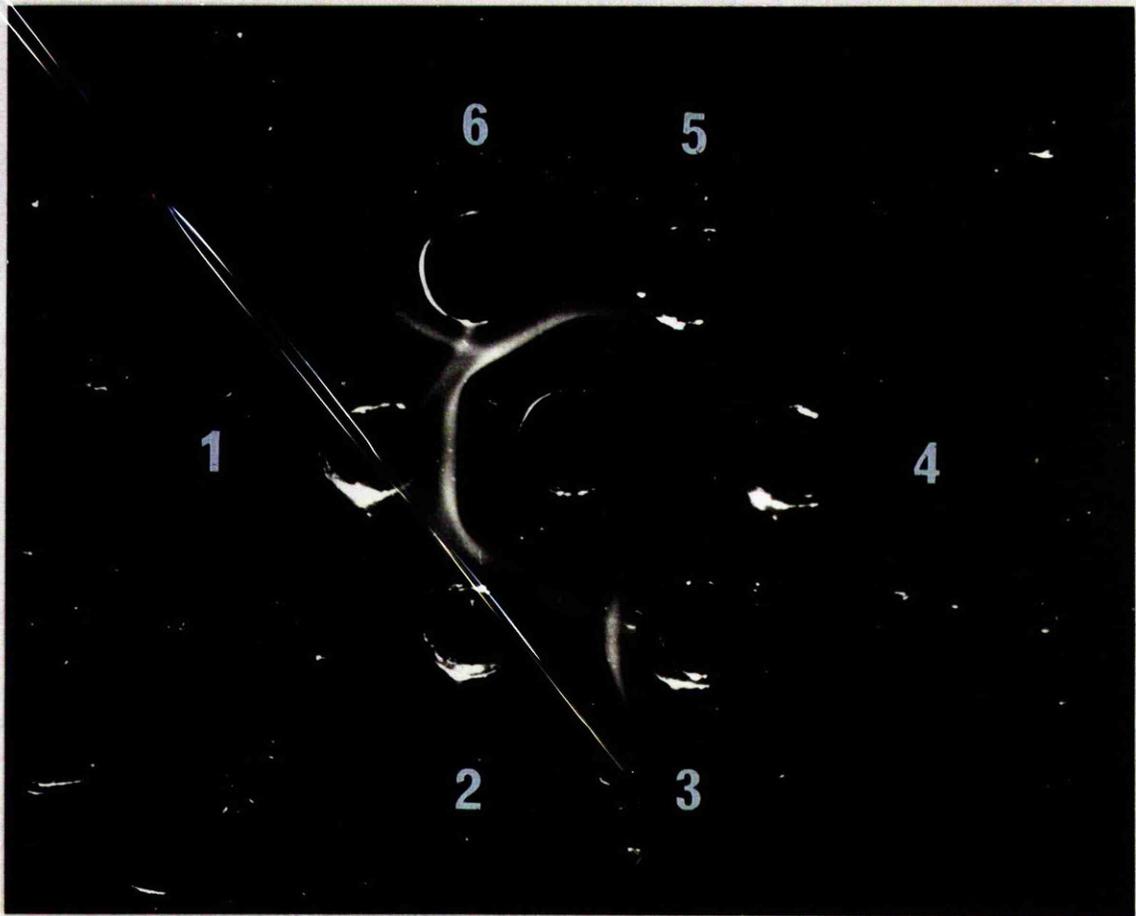
The centre well was loaded with high density lipoprotein prepared from a normal subject. The peripheral wells were loaded with

1. antiserum 81    2. antiserum 81 diluted to equivalence with saline.    3, 4, 5 and 6 supernates obtained following reaction of 0.1 ml antiserum with 0.1 ml low density lipoprotein from plasma of subject no. 16 of Mar. 7th, Mar. 11th, Mar. 20th, and Mar. 29th respectively (see Table 10.4 for corresponding plasma lipid levels).

In 1 and 2, the undiluted and diluted antiserum has reacted with the normal alpha lipoprotein/

lipoprotein to produce a precipitin line. In 3 and 4, the antiserum has not produced this line, and in 5 it has produced a faint line only. A dense precipitin line is seen in 6 indicating no significant removal of anti-alpha lipoprotein reactivity from the antiserum by the low density fraction of Mar. 29th when the plasma lipids had fallen substantially and the abnormal arc seen on immunoelectrophoresis had disappeared.

An additional precipitin line is observed between 1 and 6, and between 2 and 3; it results from antiserum 81, which has known anti-beta lipoprotein reactivity, in wells 1 and 2 reacting with the beta lipoprotein of the low density lipoprotein fraction in wells 3 and 6.



Legend for Fig. 4.15

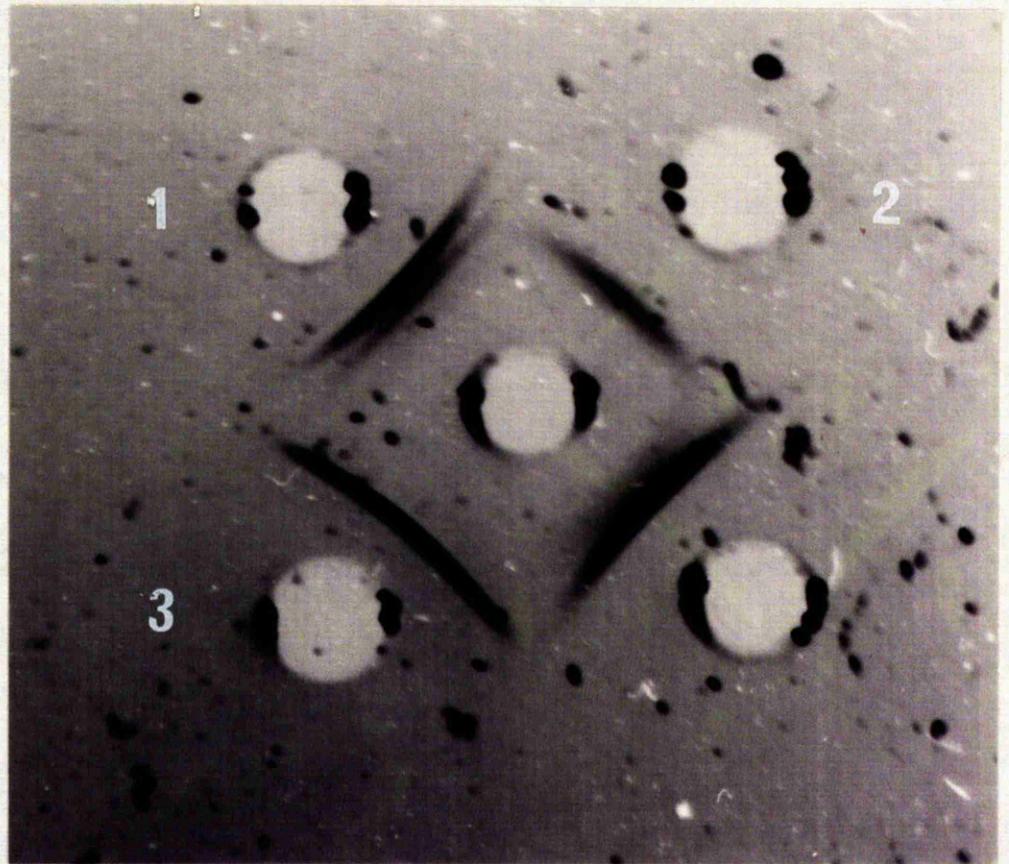
IMMUNODIFFUSION OF ETHER-DELIPIDATED  
LOW DENSITY LIPOPROTEIN FRACTION IN  
OUCHTERLONY PLATE

The antiserum in the centre well was 124A with anti-alpha and anti-beta lipoprotein reactivity. The peripheral wells were loaded with

1. ether-delipidated low density lipoprotein (subject no. 15)
2. delipidated high density lipoprotein from a normal subject.
3. low density lipoprotein from a normal subject.

In 1 there is a beta lipoprotein precipitin line giving a reaction of identity with the beta lipoprotein line in 3 (this was better seen by visual inspection of the plate). The line formerly seen peripheral to the beta lipoprotein line/

line (see Fig. 4.14) in 2 is no longer evident. The inner line in 1 gives a reaction of partial identity with the delipidated normal high density lipoprotein in 2.



Legend for Fig. 4.16

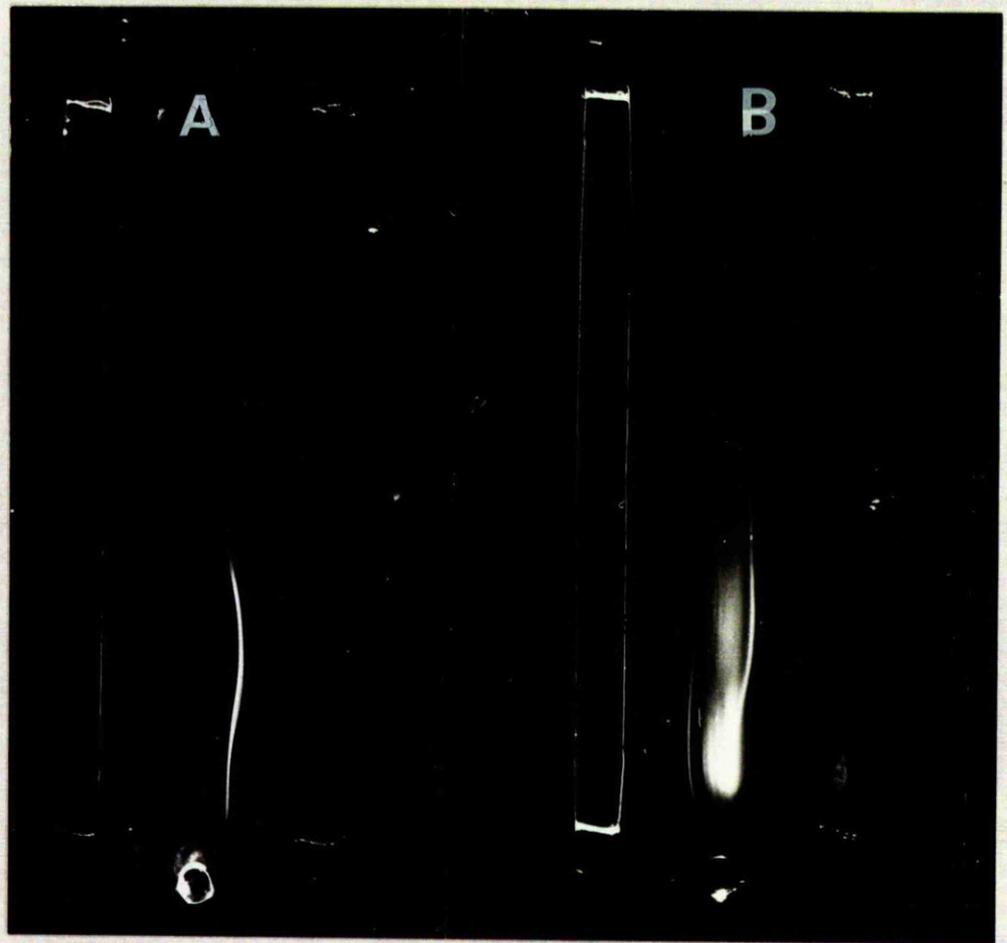
IMMUNOELECTROPHORESIS IN AGAROSE GEL OF  
ETHER-DELIPIDATED LOW DENSITY  
LIPOPROTEIN FRACTION

Plasma of subject no. 15.

The antiserum in each left-hand trough was R5 with anti-beta and anti-alpha lipoprotein reactivity, and in each right-hand trough, Hyland anti-beta which is monospecific for beta lipoprotein.

In B the native low density lipoprotein has yielded, with Hyland anti-beta, a beta lipoprotein precipitin arc and, with R5, the additional arc.

In A the delipidated low density lipoprotein fraction shows, with R5, a faint precipitin arc corresponding to the beta lipoprotein arc produced with Hyland anti-beta, and a new faint lipoprotein arc with the approximate mobility of normal alpha lipoprotein.

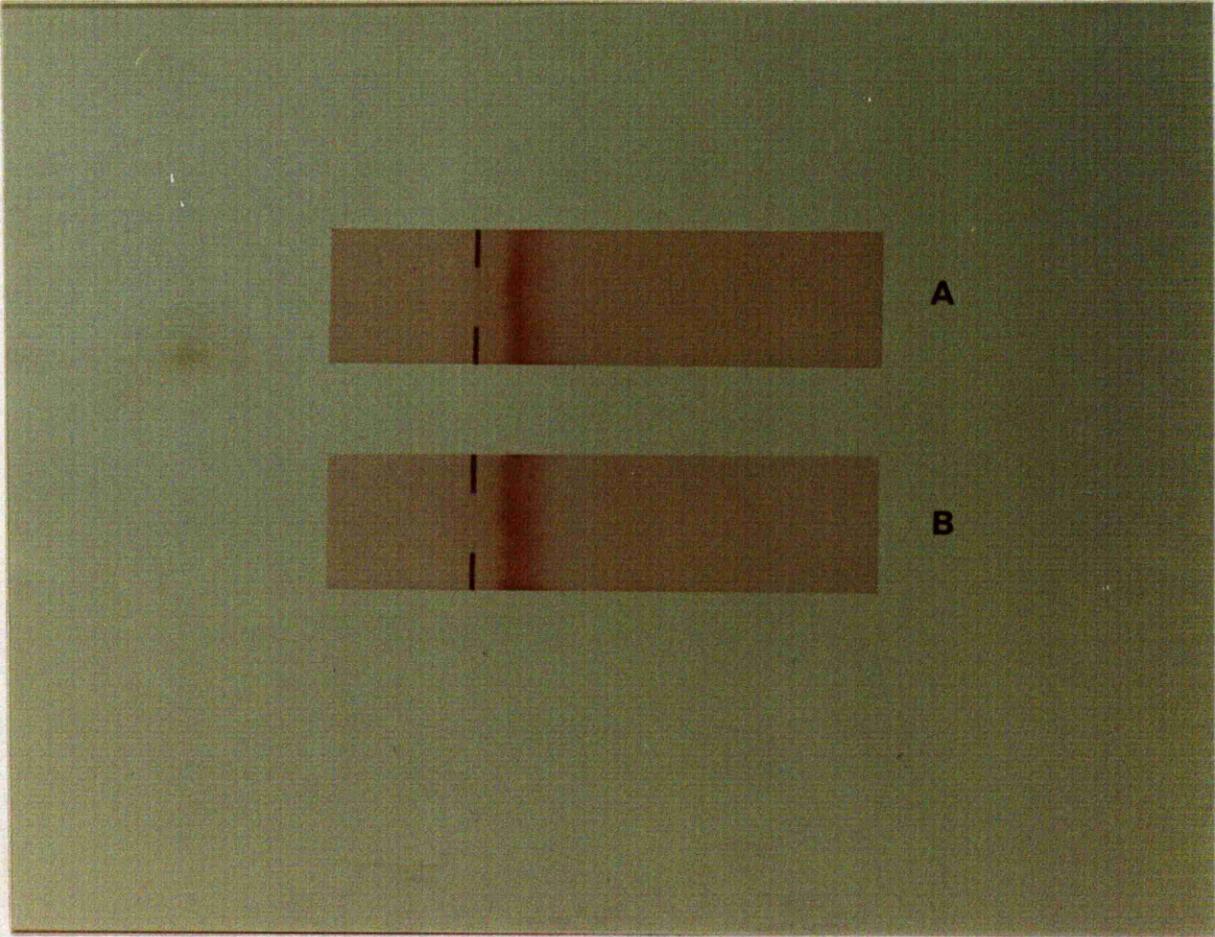


FIGURES  
CHAPTER 5

Legend for Fig. 5.1

LIPOPROTEIN PAPER ELECTROPHORESIS OF PLASMA

- A. Plasma of subject no. 14 (load 20  $\mu$ l) -- shows a slightly prominent, sharply defined beta lipoprotein band and an absent alpha lipoprotein band. Despite a triglyceride level of 298 mg/100 ml, there is only a slight pre-beta lipoprotein band.
- B. Plasma of subject no. 17 (load 20  $\mu$ l) - shows a beta lipoprotein band of increased intensity and breadth, and of decreased definition resembling the "broad" beta band of Type III hyperlipoproteinaemia (triglyceride level 249 mg/100 ml). The reproduction barely shows the faint alpha lipoprotein band which was present in the original paper strip.



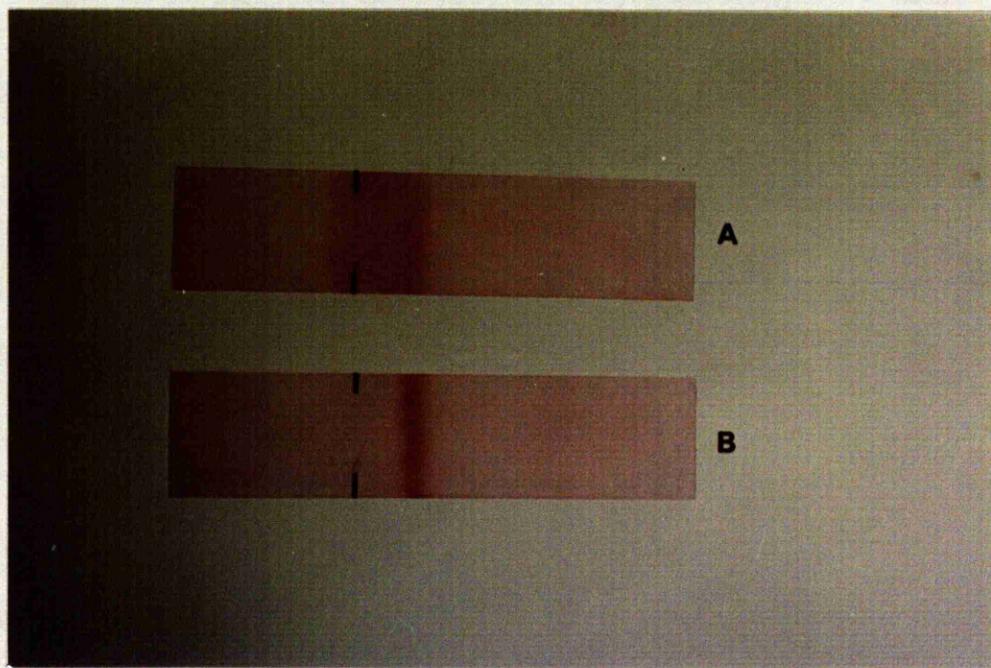
Legend for Fig 5.2

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF  $d < 1.006$  AND  $d > 1.006$  FRACTIONS

Fractions of subject no. 14.

- A.  $d < 1.006$  fraction          Load 40  $\mu$ l  
B.  $d > 1.006$  fraction          Load 20  $\mu$ l

The  $d < 1.006$  fraction contains lipo-  
protein of similar mobility to the beta lipo-  
protein in the  $d > 1.006$  fraction. Some  
"trail" is seen running from it to the origin.



**A**

**B**

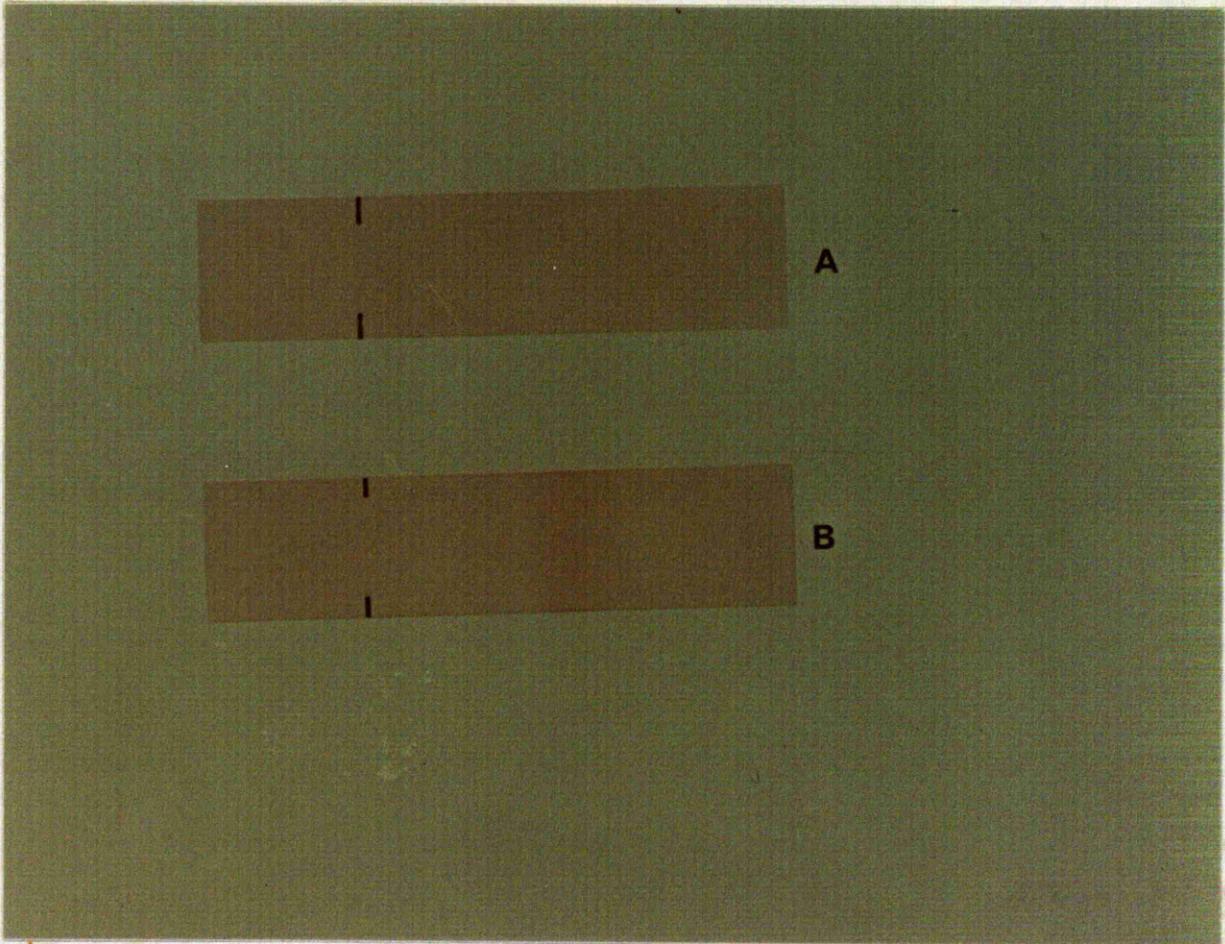
Legend for Fig. 5.3

LIPOPROTEIN PAPER ELECTROPHORESIS

OF  $d > 1.063$  FRACTION

A Subject no. 14 (load 40  $\mu$ l) - shows absence  
of alpha lipoprotein band.

B Subject no. 17 (load 40  $\mu$ l) - shows faint  
alpha lipoprotein band.

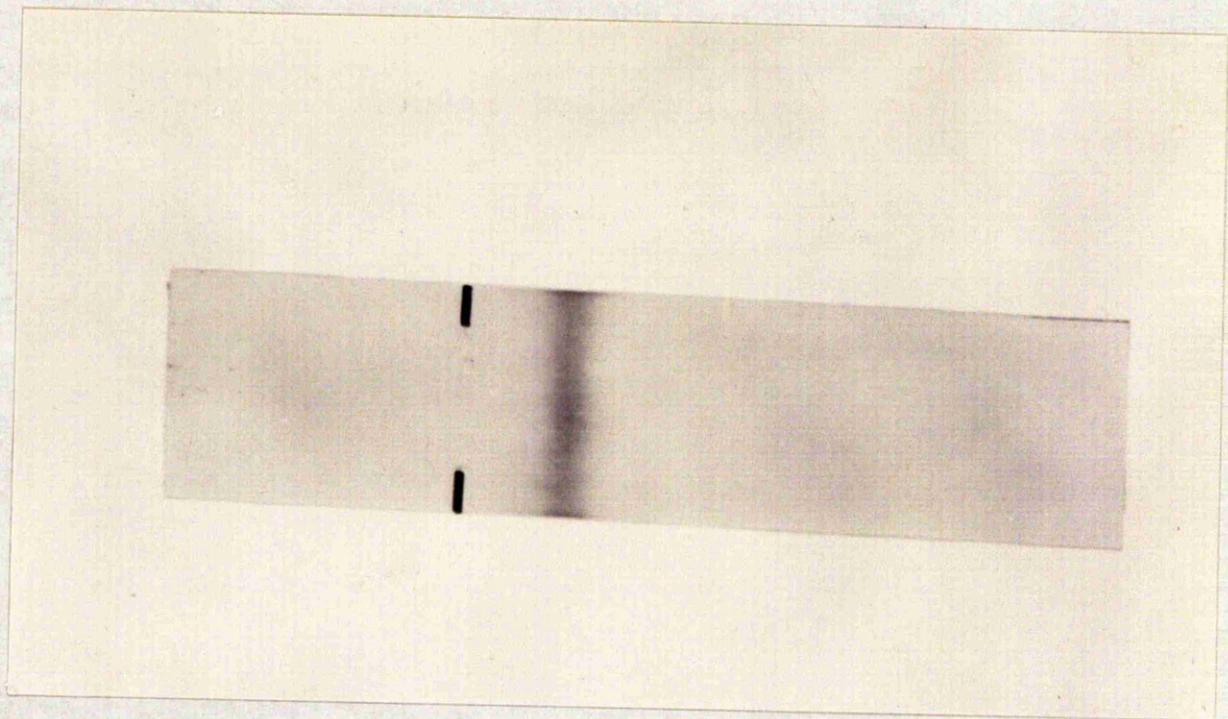


Legend for Fig. 5.4

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF LOW DENSITY LIPOPROTEIN FRACTION

Subject no. 17                      Load 40  $\mu$ l

A single well-defined beta lipoprotein  
band is present.



Legend for Fig. 5.5a and b

PAPER ELECTROPHORESIS OF LOW DENSITY

LIPOPROTEIN FRACTION

DURING AND AFTER JAUNDICE

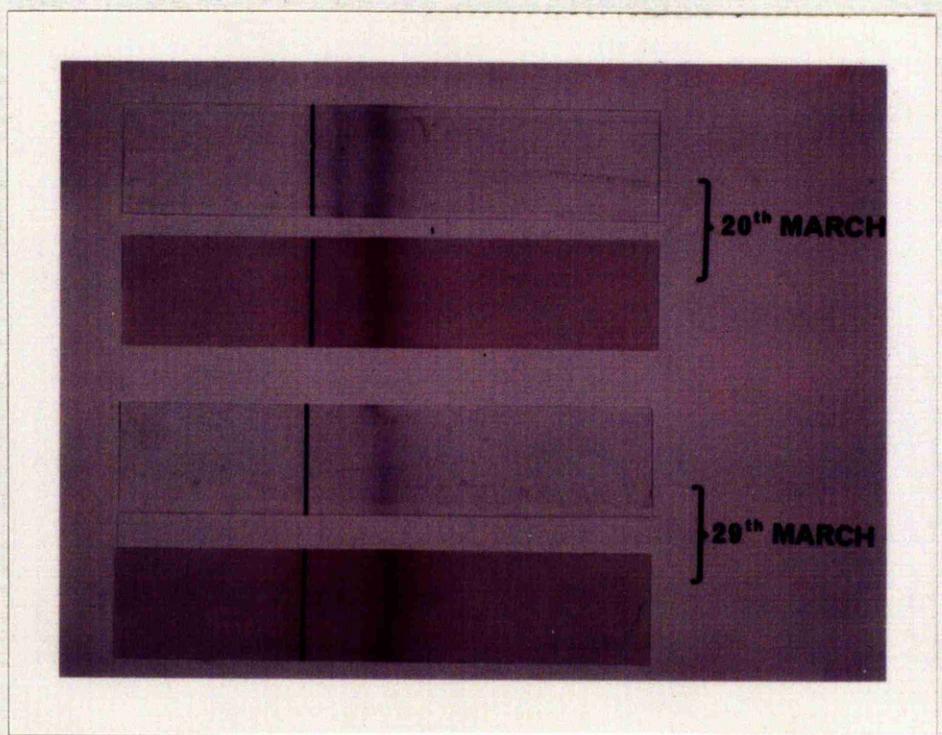
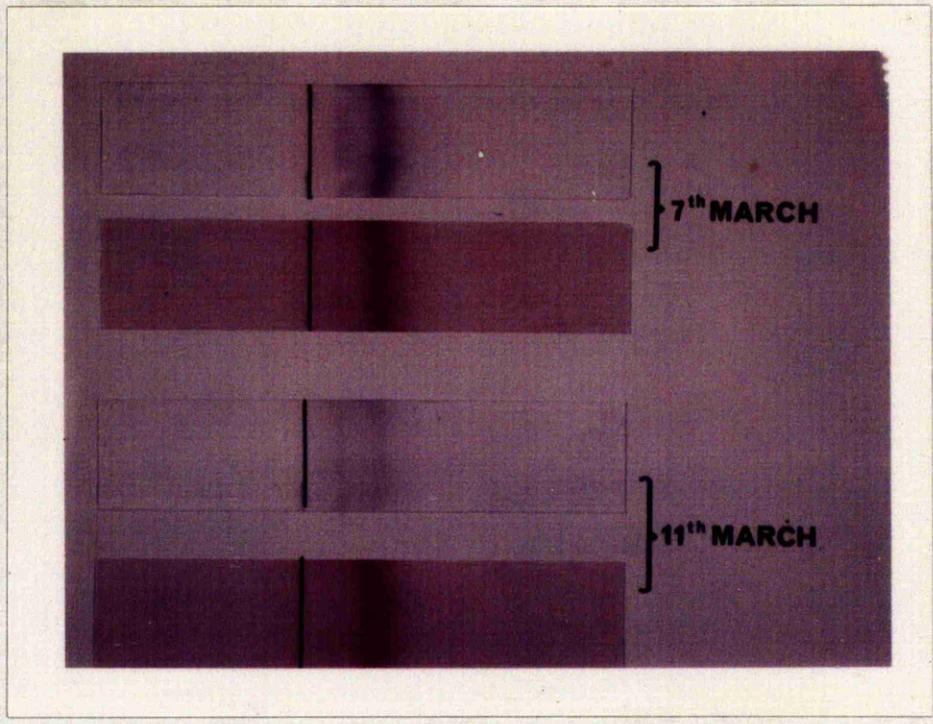
Fractions of subject no. 16 obtained during (Mar. 7th, 11th, 20th) and after (Mar. 29th) jaundice.

The barbital buffer did not contain albumen to permit staining for protein. The upper strip of each pair was stained with Oil red O and the lower with bromophenol blue.

The samples obtained during the jaundiced phase all show a single beta lipoprotein band with the lipid stain, and an additional slower-migrating band with the protein stain. This band was subsequently shown to contain lipid.

The sample obtained following recovery from the jaundice showed only a beta lipoprotein band with either stain.

The omission of albumen from the buffer has resulted in bands which are not sharply defined.



Legend for Fig. 5.6

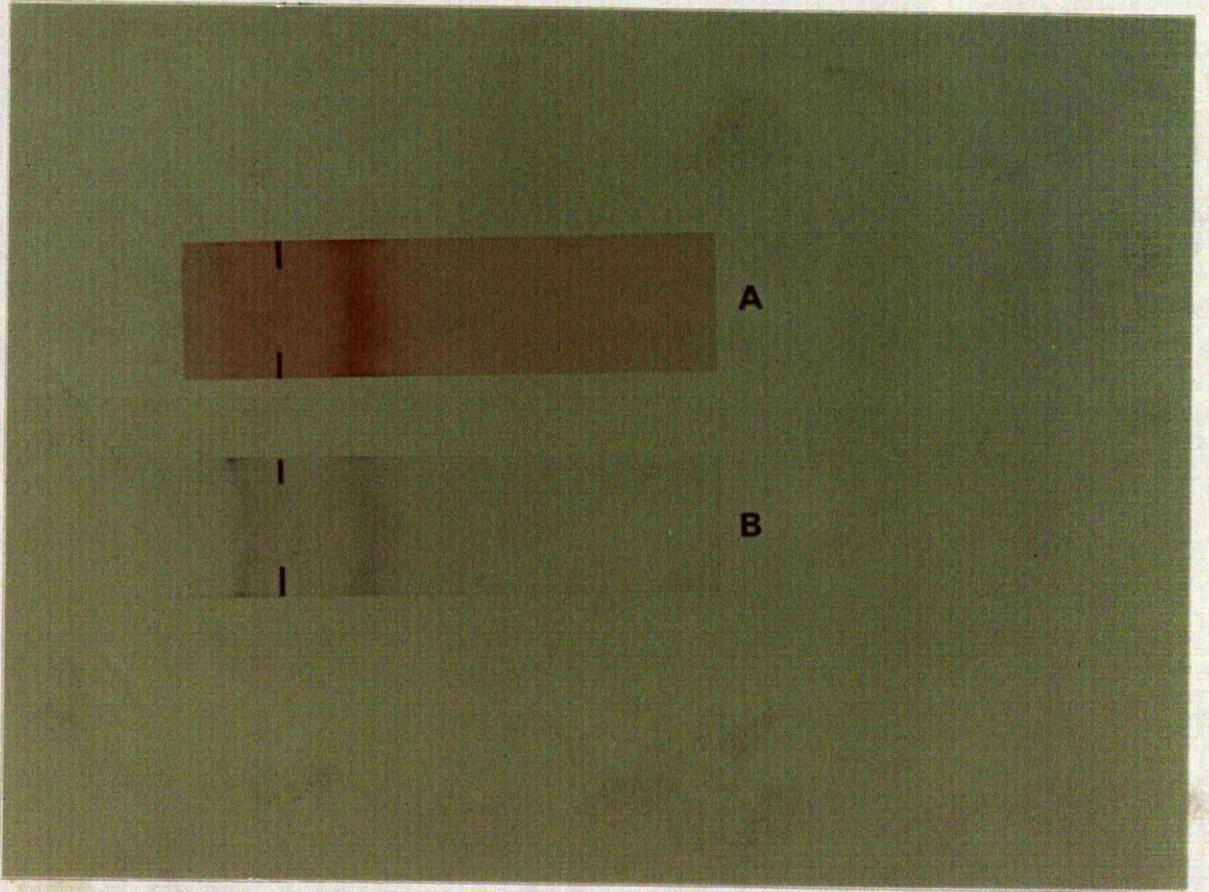
PAPER ELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION

Fraction of subject no. 15.

Albumen was omitted from the barbital buffer to permit staining for protein. Corresponding strips, each of which had been loaded with 40  $\mu$ l of the fraction, were stained with A Oil red O, and B bromophenol blue.

In addition to a beta-migrating band produced with either stain, a cathodal-migrating band is seen. It stained better for protein than for lipid.

The omission of albumen from the buffer has resulted in bands which are not sharply defined.



**A**

**B**

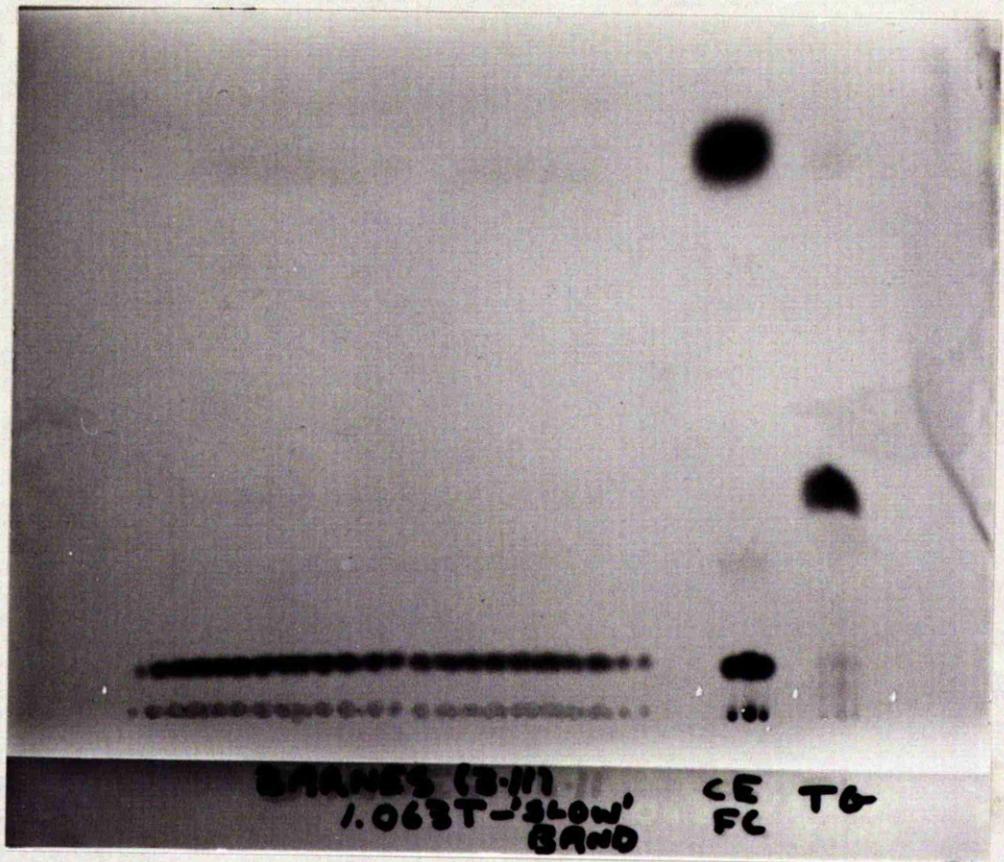
Legend for Fig. 5.7

THIN LAYER CHROMATOGRAM OF NON-POLAR LIPIDS  
OF EXTRACT OF "SLOW" BAND  
FROM LOW DENSITY LIPOPROTEIN FRACTION

The non-polar lipid standards were -  
CE = cholesterol ester, FC = free cholesterol,  
TG = triglyceride.

The slow band from the low density lipoprotein fraction of subject no. 16 (Mar. 11th) contained non-polar lipid, predominantly free cholesterol together with a faint amount of cholesterol ester. In the original plate a very faint amount of triglyceride was also seen.

Details of the non-polar solvent system are given in Chapter 3. The plates were stained with anisaldehyde.



SPANS (B-M)  
1.063T-SLOW  
BAND

CE TO  
FL

Legend for Fig. 5.8

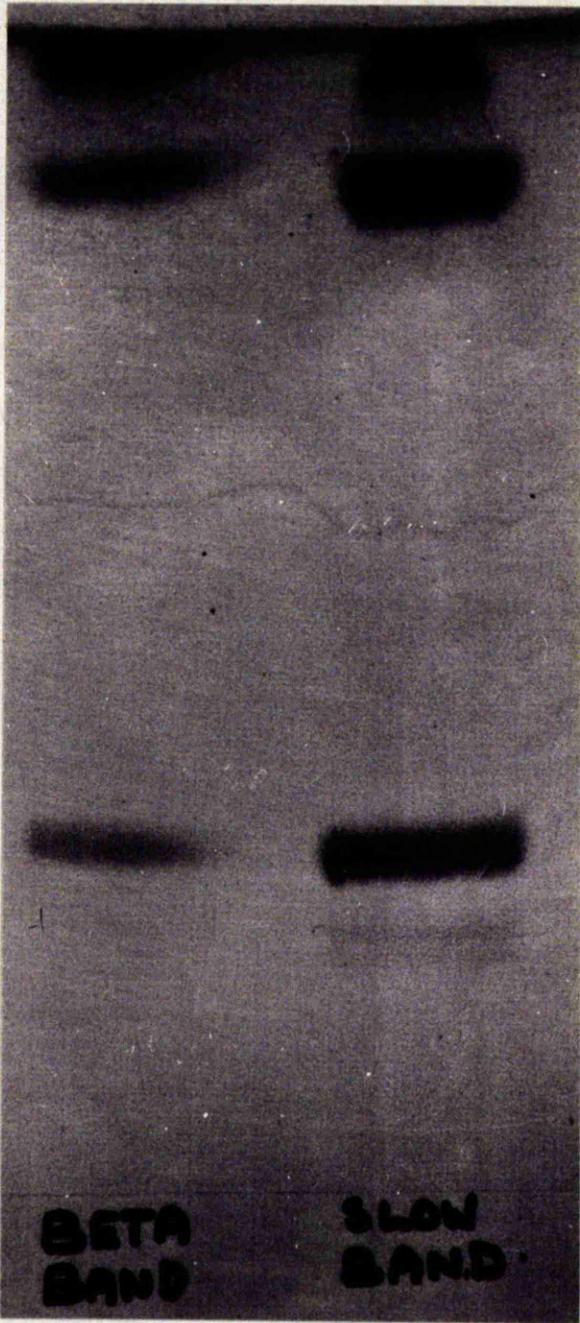
THIN LAYER CHROMATOGRAM OF NON-POLAR LIPIDS  
OF EXTRACT OF "SLOW" AND BETA BANDS  
FROM LOW DENSITY LIPOPROTEIN FRACTION

Fraction of subject no. 16 (Mar. 11th).

L = lecithin            S = sphingomyelin

The slow band from the low density lipoprotein fraction produced a prominent lecithin and a faint sphingomyelin spot. The beta band produced a weaker lecithin spot and a very faint sphingomyelin spot, which does not show up in the print. The spots at the top of the plate are due to non-polar lipid. Polar lipid standards were concurrently run.

Details of the polar solvent system are given in Chapter 3. The plates were stained with anisaldehyde.



BETA  
BAND

SLOW  
BAND

▲ L  
▲ S

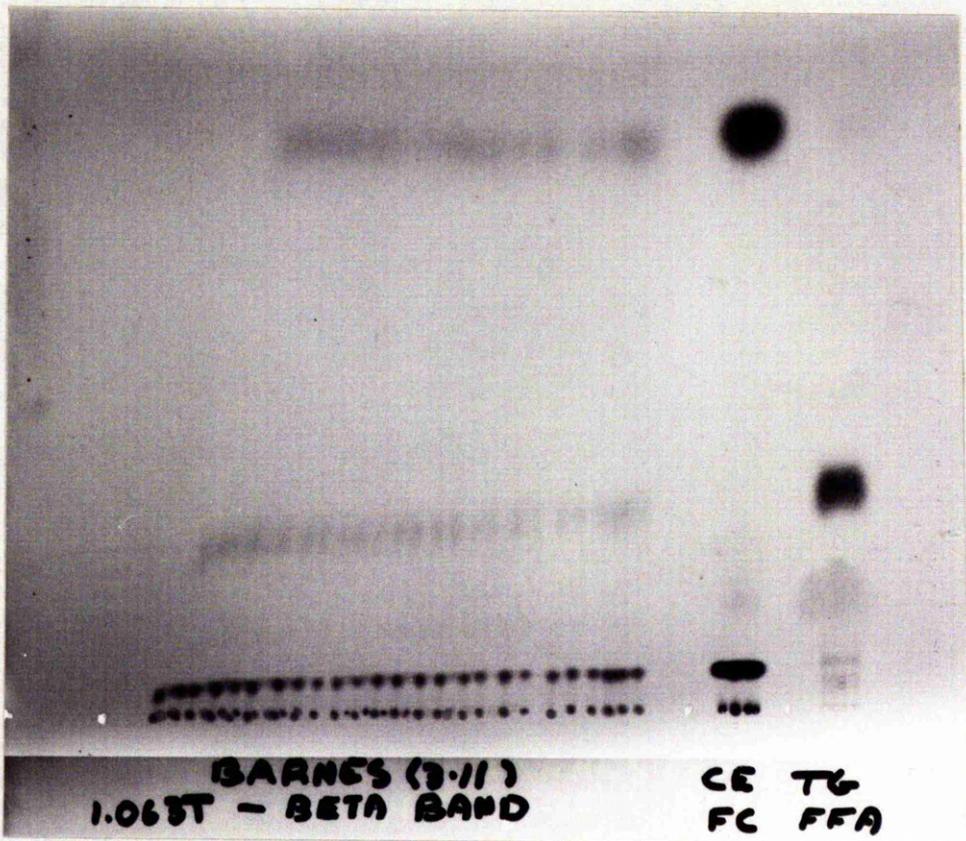
Legend for Fig. 5.9

THIN LAYER CHROMATOGRAM OF NON-POLAR LIPIDS  
OF EXTRACT OF BETA LIPOPROTEIN BAND  
FROM LOW DENSITY LIPOPROTEIN FRACTION

The non-polar lipid standards were -  
CE = cholesterol ester, FC = free cholesterol,  
TG = triglyceride, FFA = free fatty acid.

The beta band from the low density lipoprotein fraction of subject no. 16 (Mar. 11th) contained non-polar lipid consisting of cholesterol ester, free cholesterol and triglyceride.

Details of the non-polar solvent system are given in Chapter 3. The plates were stained with anisaldehyde.



BARNES (3-11)  
1.063T - BETA BAND

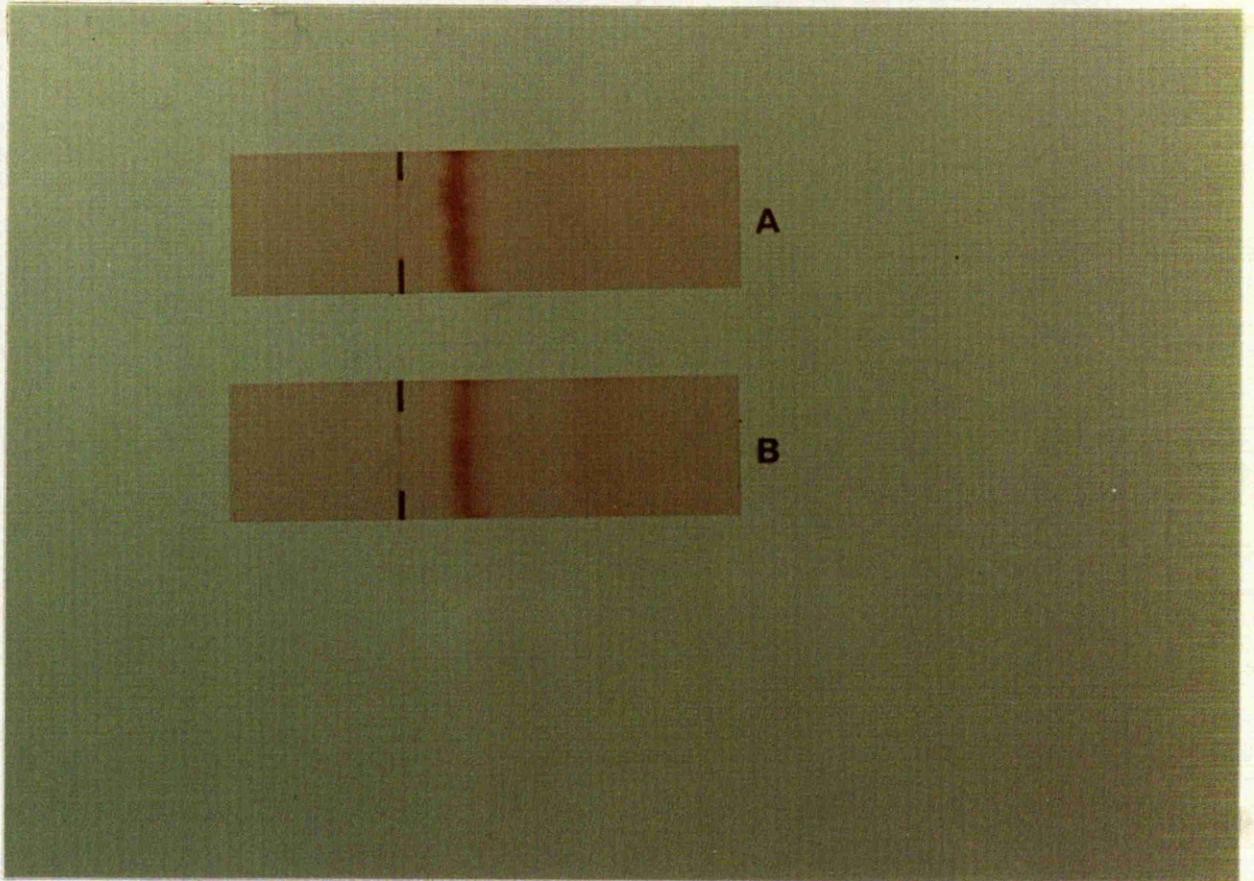
CE TG  
FC FFA

FIGURES  
CHAPTER 7

Legend for Fig. 7.1

LIPOPROTEIN PAPER ELECTROPHORESIS OF PLASMA  
FROM SUBJECTS WITH OBSTRUCTIVE JAUNDICE

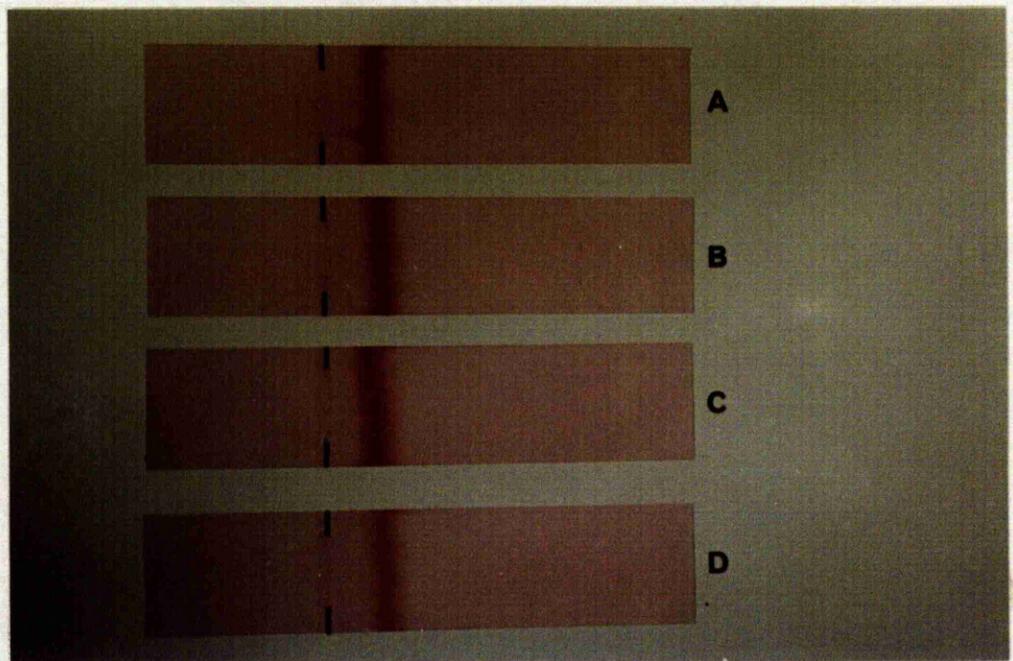
The strips from A subject no. 1 and B subject no 5 were each loaded with 20  $\mu$ l of plasma. They show lipoprotein bands of beta mobility which are of increased or normal intensity respectively. They show either faint or absent bands with the mobility of alpha lipoprotein.



Legend for Fig. 7.2

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF LOW DENSITY LIPOPROTEIN FRACTION  
FROM 3 SUBJECTS WITH OBSTRUCTIVE JAUNDICE  
AND A CONTROL SUBJECT

A, B and C. Each was loaded with 40  $\mu$ l of low density lipoprotein fraction from subjects no. 1, 2 and 3 respectively. Each shows a single intense lipoprotein band of beta mobility which may be contrasted with D. the single less intense band from the fraction of the control subject. Since twice the usual load was applied, to reveal any abnormal lipoprotein band that might be present, the beta bands are more intense than usual.



Legend for Fig. 7.3

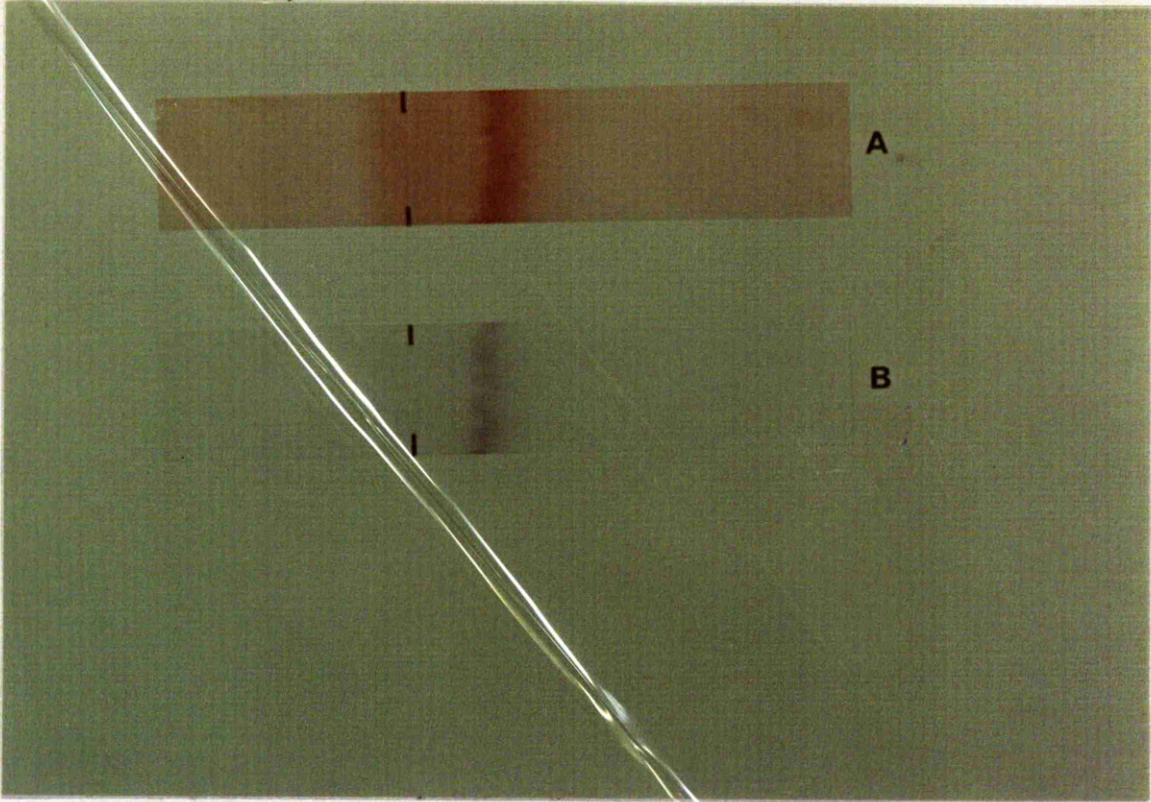
PAPER ELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION

Fraction of subject no. 9.

Albumen was omitted from the barbital buffer to permit protein staining.

Corresponding strips, each of which had been loaded with 40  $\mu$ l of the low density lipoprotein fraction, were stained with A Oil red O, or B bromophenol blue.

A single band of beta mobility is shown by either stain. The decreased sharpness of the bands is attributable to the omission of the albumen from the buffer.



A

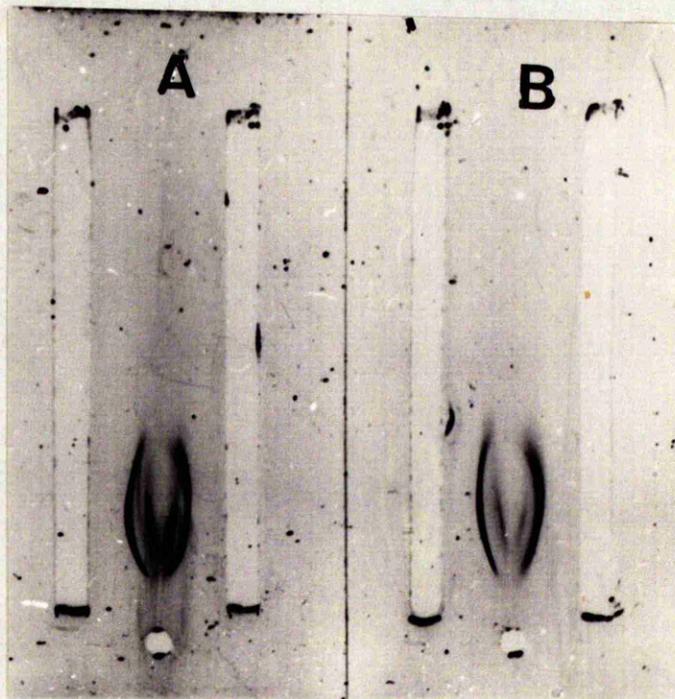
B

Legend for Fig. 7.4

IMMUNOELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION IN AGAROSE GEL FROM  
SUBJECTS WITH OBSTRUCTIVE JAUNDICE

Fraction from A subject no. 2, and B  
subject no. 3.

The antiserum in each left-hand trough  
was 79, and in each right-hand trough 81.  
A lipoprotein precipitin line of beta mob-  
ility is observed in each case.



Legend for Fig. 7.5

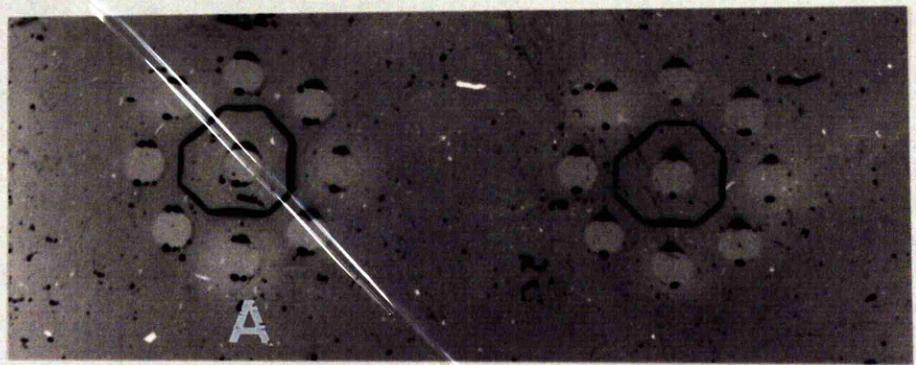
IMMUNODIFFUSION IN OUCHTERLONY PLATE OF  
LOW DENSITY LIPOPROTEIN FRACTION FROM  
SUBJECTS WITH OBSTRUCTIVE JAUNDICE

The antiserum in the centre well in the left-hand set of wells was 124A, and in the right-hand set, 360.

Each set of peripheral wells were loaded with the low density fraction from the following subjects commencing with A and moving in a clockwise direction.

1. subject no. 1; 2. subject no. 2;
3. normal beta lipoprotein; 4. subject no. 3;
5. normal beta lipoprotein; 6. B.R. (control subject);
7. subject no. 5; and 8. normal beta lipoprotein.

Each low density lipoprotein sample yielded with either antiserum a single precipitin line which gave a reaction of identity with that from the normal beta lipoprotein.



Legend for Fig. 7.6

IMMUNODIFFUSION IN AGAR GEL OF LOW DENSITY  
LIPOPROTEIN FRACTION SHOWING REMOVAL OF BETA  
LIPOPROTEIN BY ANTI-BETA LIPOPROTEIN ANTISERA

Each set of peripheral wells was loaded as follows commencing with well A and moving in an anti-clockwise direction.

- A low density lipoprotein fraction of given subject;
- B 0.1 ml low density fraction diluted with 0.1 ml EDTA-saline;
- C, D and E supernates from 0.1 ml low density fraction reacted with 0.03 ml, 0.08 ml or 0.10 ml respectively of a gamma-globulin preparation of antiserum 360;
- F, G and H similar samples in which a gamma-globulin preparation of antiserum 124A replaced the gamma-globulin preparation of antiserum 360.

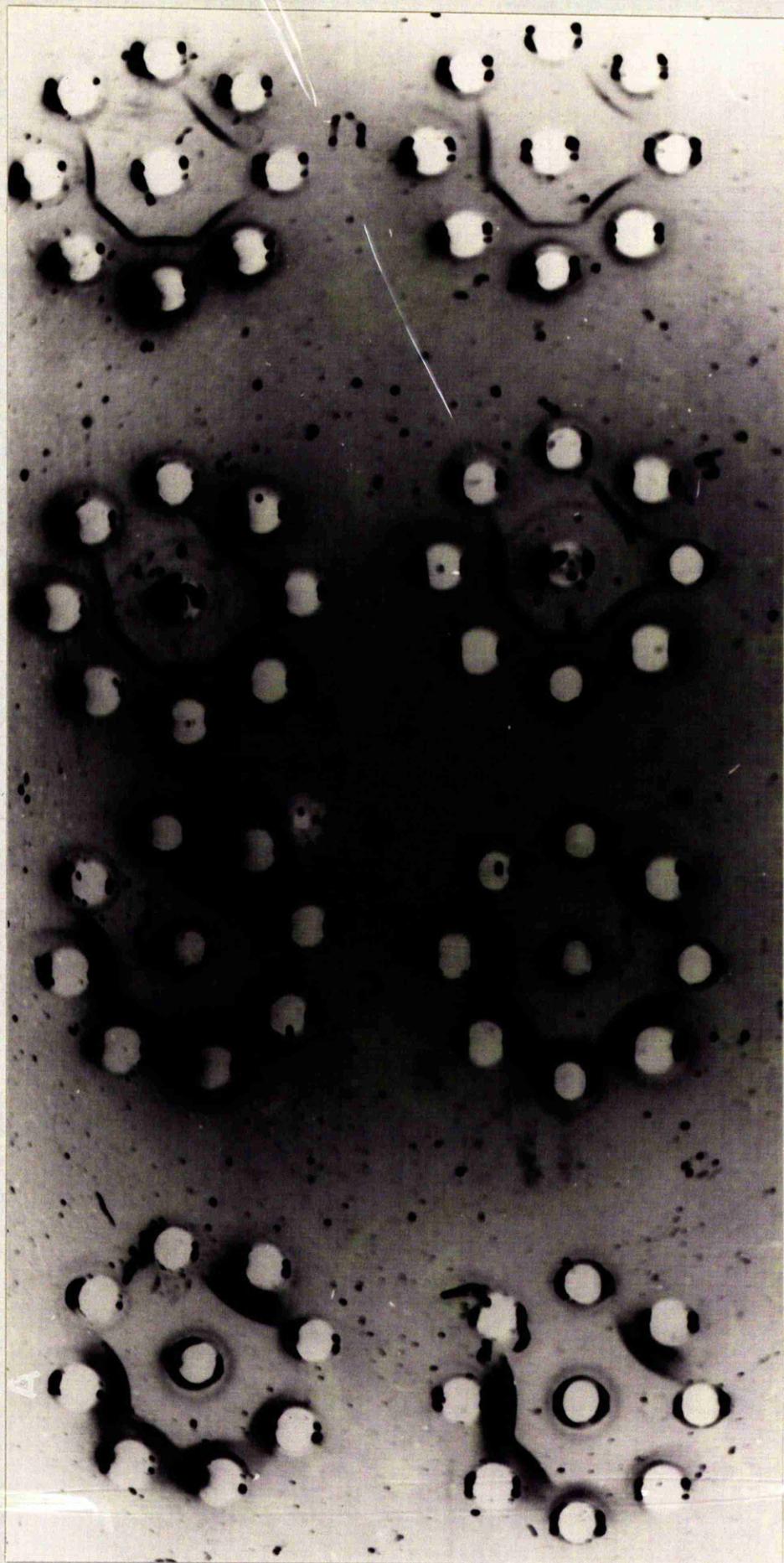
The/

The four sets of wells occupying a horizontal row were used to study a given subject.

The subjects studied were row 1. no. 2, row 2. no. 3.

The antisera in the centre wells in a given row from left to right were 124A, 360, AHS, and 79.

The undiluted and saline-diluted low density lipoprotein fractions of each of the subjects has resulted in a beta lipoprotein precipitin line with each of the antisera. This line has not been produced by the supernates from the fractions previously reacted with the maximum amounts of either of the antisera.



Legend for Fig. 7.7

IMMUNOELECTROPHORESIS IN AGAR GEL SHOWING  
ABSENCE OF BETA LIPOPROTEIN PRECIPITIN LINE  
FROM LOW DENSITY FRACTION FOLLOWING REACTION  
WITH ANTI-BETA LIPOPROTEIN ANTISERA

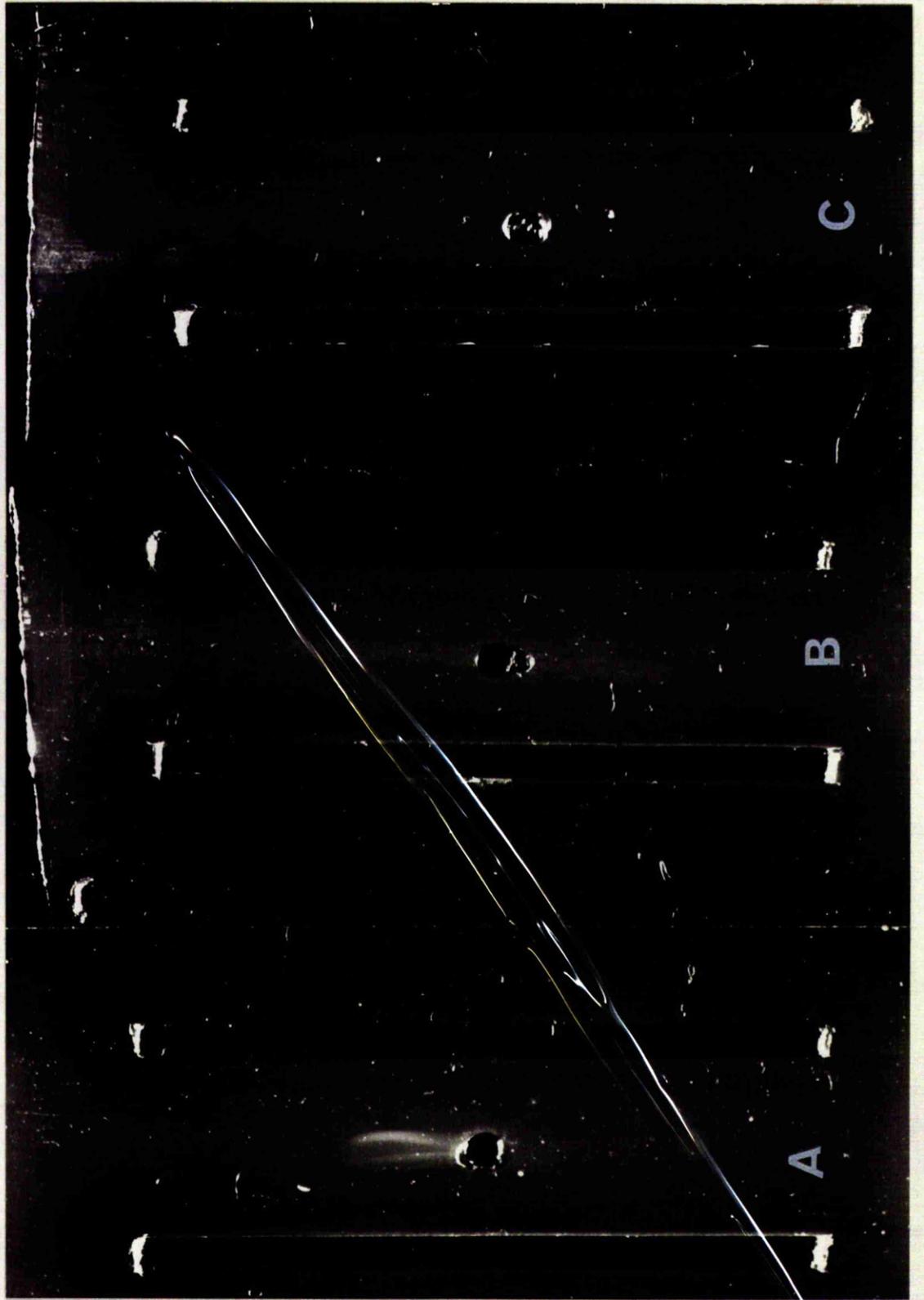
The centre wells were loaded with

- A - low density lipoprotein fraction diluted  
with 3 volumes of EDTA-saline from sub-  
ject no. 8 from Group I (see text for  
definition of Group I).
- B - supernate from this fraction reacted  
with 3 volumes of antiserum 360 (anti-  
beta lipoprotein antiserum).
- C - supernate from this fraction reacted  
with 3 volumes of antiserum 124 (anti-  
alpha lipoprotein, anti-beta lipoprotein  
and anti-albumen antiserum).

All left-hand troughs were loaded with  
antiserum R5 and all right-hand troughs with  
Hyland/

Hyland anti-beta lipoprotein antiserum.

In A a beta lipoprotein precipitin arc, of reduced intensity because of the saline dilution, is seen with either antiserum. The arc is not seen in B or C indicating its prior removal by antisera 360 or 124.

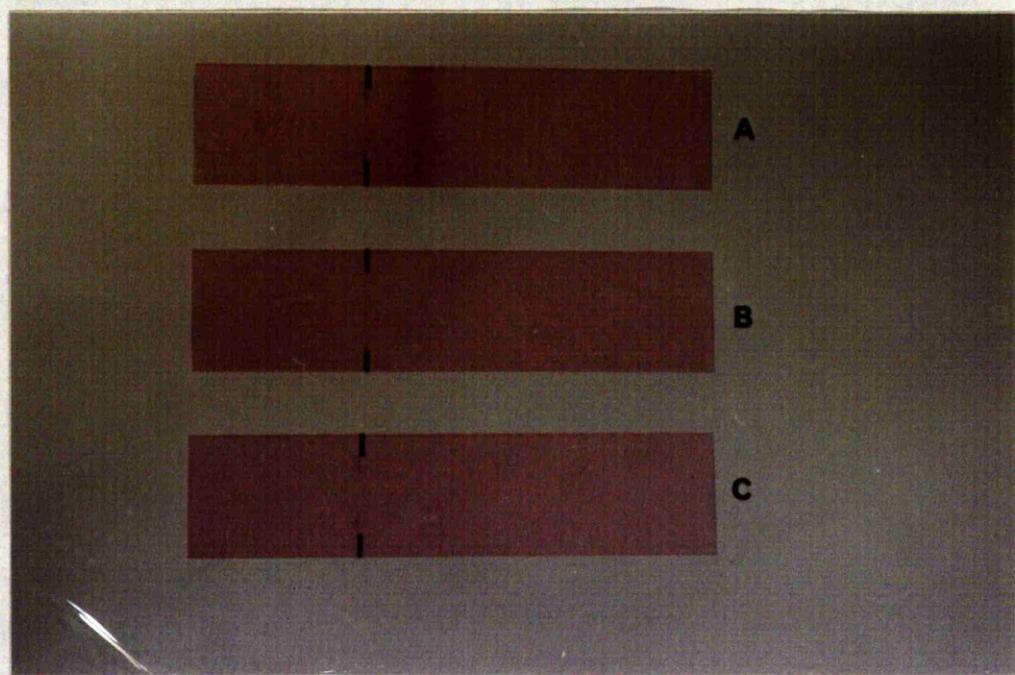


Legend for Fig. 7.8

PAPER ELECTROPHORESIS OF LOW DENSITY FRACTION  
SHOWING ABSENCE OF BETA LIPOPROTEIN BAND  
FOLLOWING REACTION WITH ANTI-BETA  
LIPOPROTEIN ANTISERA

Fraction of subject no. 3.

In A. the fraction has resulted in a normal beta lipoprotein band. This band is not present in either B. or C. which represent the fractions following their absorption with antisera 124A or 360 respectively.



Legend for Fig. 7.9a and b

THIN LAYER CHROMATOGRAM SHOWING REMOVAL OF  
NON-POLAR LIPIDS FROM LOW DENSITY FRACTION  
FOLLOWING ITS REACTION WITH ANTI- $\alpha$   
AND ANTI- $\beta$  LIPOPROTEIN ANTISERUM

The subjects were 1. no. 1; 2. no. 2;  
3. no. 3 (all from Group I as defined in  
text); 4. B (control); and 5. no. 11  
(from Group II).

A refers to sample derived from chloro-  
form:methanol extract of supernate from 0.3  
ml low density lipoprotein fraction reacted  
with gamma-globulin preparation of antiserum  
124A, except for no. 2 where 0.5 ml of anti-  
serum was used.

O refers to low density lipoprotein  
fraction diluted with corresponding volume of  
EDTA-saline.

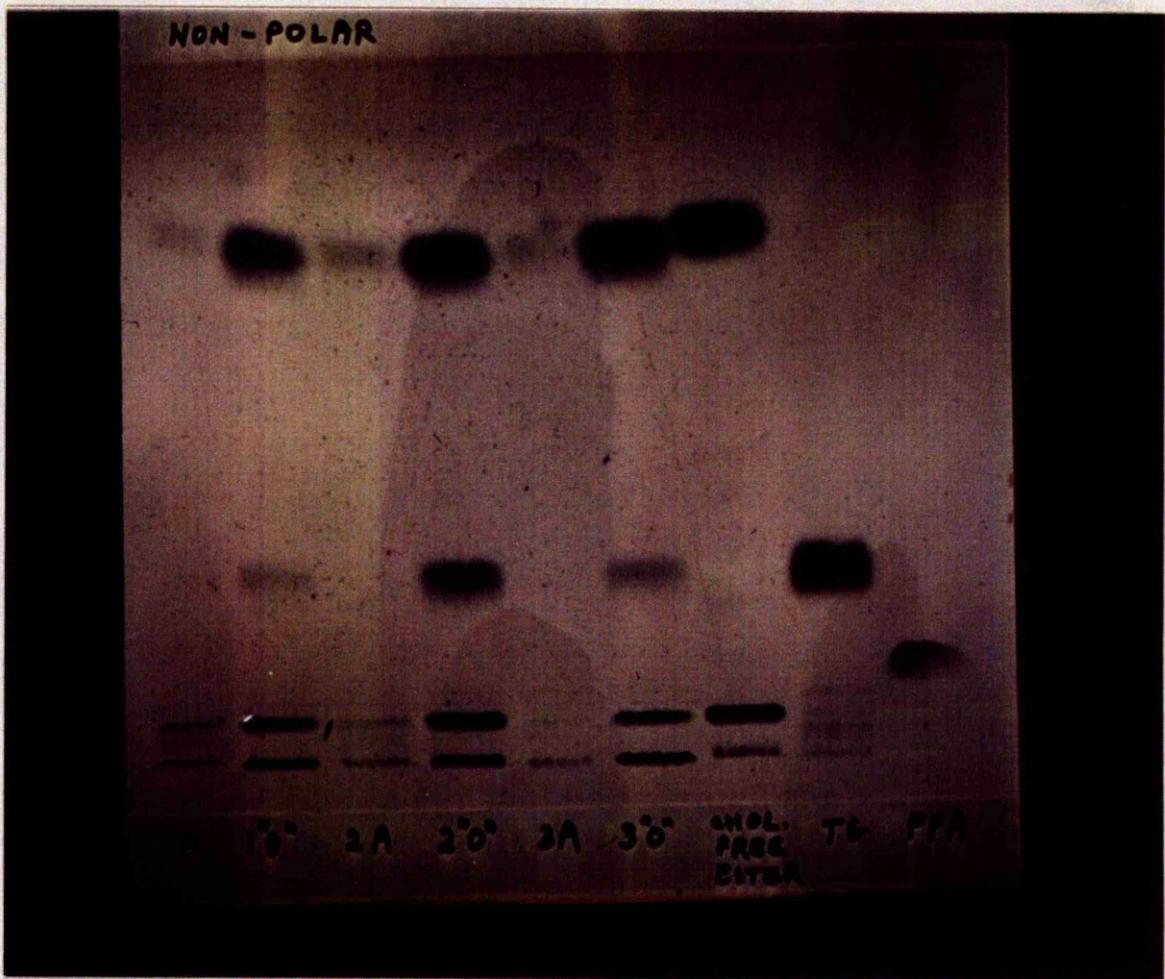
Extracts of the corresponding EDTA-saline  
dilutions of the antiserum (designated  $\begin{matrix} .30 \\ .3A \end{matrix}$  and  
 $\begin{matrix} .30 \\ .5A \end{matrix}$ )

<sup>30</sup><sub>5A</sub>) were also run, as were the following non-polar lipid standards. FFA = free fatty acid, TG = triglyceride, chol = cholesterol (free and ester).

All extracts from saline diluted low density fractions show free and ester cholesterol, and triglyceride spots. The triglyceride spots were faint in 4 and 5 and barely show up in the colour print.

The extracts from the antibody-treated low density fraction show removal of most of this lipid. The faint free and ester cholesterol spots which remain in 1A, 3A and 5A are of similar intensity to those of the control subject (4A) and are found in the extract of the EDTA-saline diluted antisera (<sup>30</sup><sub>3A</sub>); the spots remaining in 2A are similar to those in the diluted antiserum (<sup>30</sup><sub>5A</sub>) which contains a larger proportion of the antiserum.

NON-POLAR



1-13-67  
NON-POLAR



Legend for Fig. 7.10a and b

THIN LAYER CHROMATOGRAM SHOWING REMOVAL OF  
POLAR LIPIDS FROM LOW DENSITY FRACTION  
FOLLOWING ITS REACTION WITH ANTI-ALPHA  
AND ANTI-BETA LIPOPROTEIN ANTISERUM

The subjects were 1. no. 1; 2. no. 2;  
3. no. 3 (all from Group I as defined in text);  
4. B (control); and 5. no. 11 (from Group II).

A refers to sample derived from chloro-  
form:methanol extract of supernate from 0.3 ml  
low density fraction reacted with 0.3 ml anti-  
serum 124A, except in 2 where 0.5 ml of anti-  
serum was used.

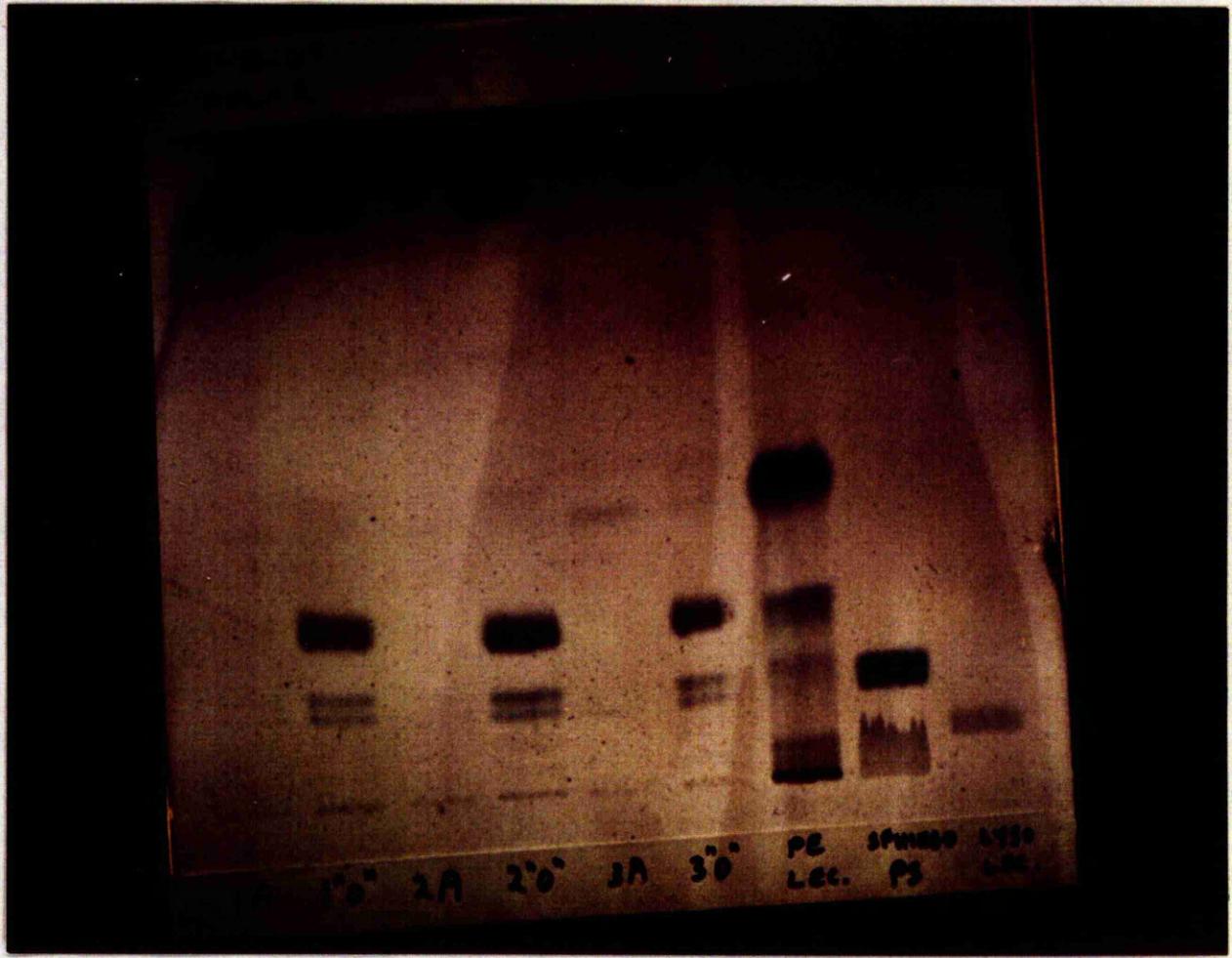
0 refers to similar extract of low  
density fraction in which EDTA-saline had been  
added instead of the antiserum.

Extracts of corresponding EDTA-saline  
dilutions of the antiserum (designated  $\cdot_{30}$  and  
 $\cdot_{3A}$  and  $\cdot_{50}$   
 $\cdot_{5A}$ ) were also run, as were the following  
polar/

polar lipid standards: lysolec = lysolecithin, sphingo = sphingomyelin, PS = phosphatidyl serine, PE = phosphatidyl ethanolamine, lec = lecithin.

The extracts from the saline-diluted low density fractions show lecithin, sphingo- myelin, and phosphatidyl ethanolamine spots.

The extract from the low density fraction reacted with the antiserum show no lecithin or sphingomyelin spots; very faint phosphatidyl ethanolamine spots remain; a similar faint spot has been produced by one of the saline-diluted antisera ( $\begin{smallmatrix} .30 \\ .5A \end{smallmatrix}$ ), and was also seen with the other diluted antiserum ( $\begin{smallmatrix} .30 \\ .3A \end{smallmatrix}$ ) although this has not shown up in the photograph. In addition, the control low density fraction (4) also showed a faint PE spot after treatment with the antiserum.





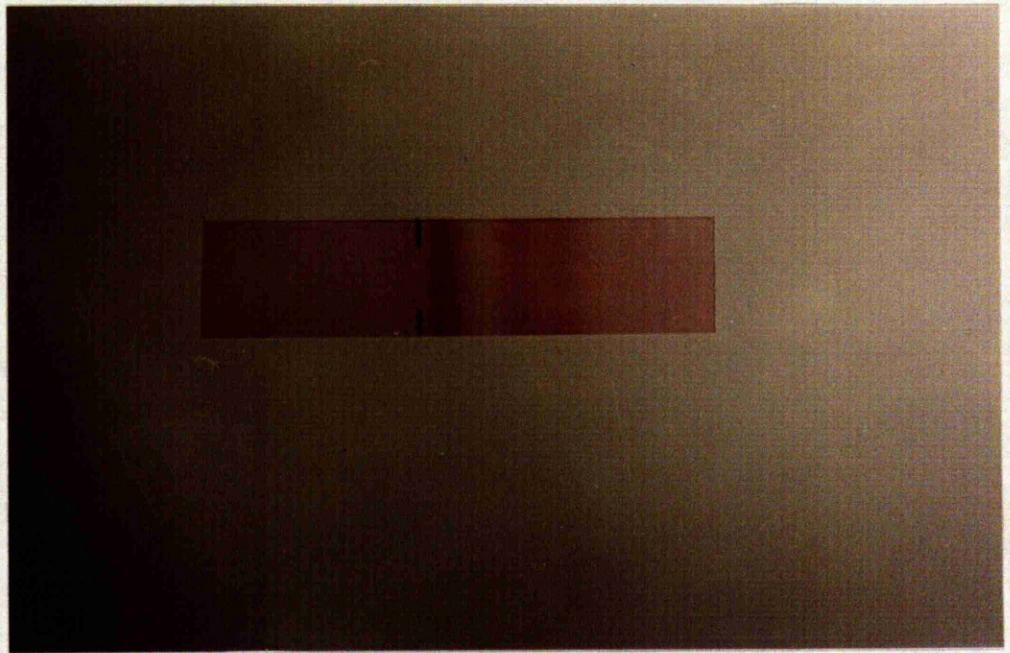
FIGURES  
CHAPTER 9

Legend for Fig. 9.1

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF PLASMA

Sample of subject no. 11.

The strip shows an increased amount of  
lipoprotein of  $\alpha_1$  mobility tending to form  
2 bands.

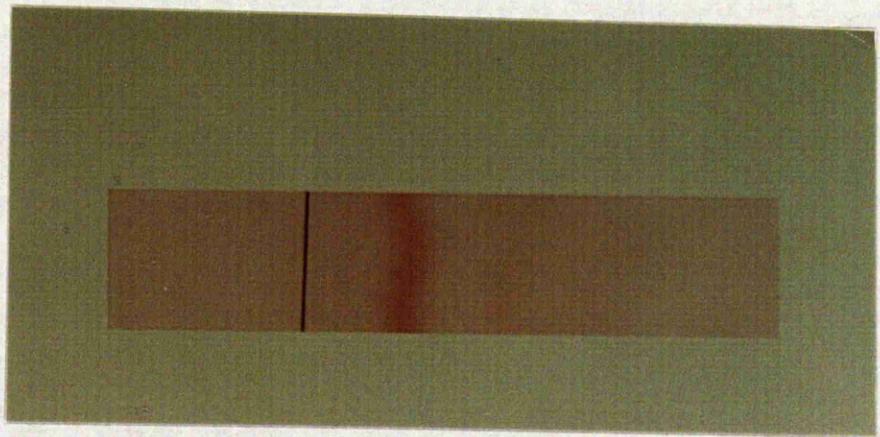


Legend for Fig. 9.2

PAPER ELECTROPHORESIS OF LOW DENSITY  
LIPOPROTEIN FRACTION

Fraction of subject no. 12.

In addition to a normal beta lipoprotein  
band a faint band of approximate  $\alpha_1$   
mobility is present.

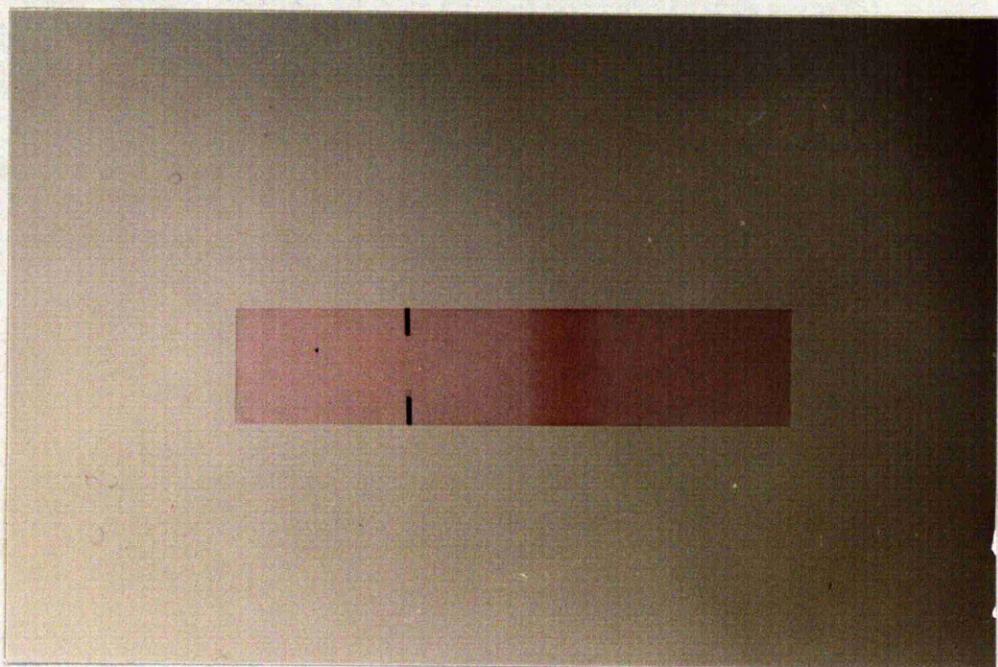


Legend for Fig. 9.3

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF HIGH DENSITY LIPOPROTEIN FRACTION

Fraction of subject no. 11.

The paper strip shows a pronounced alpha  
lipoprotein band.



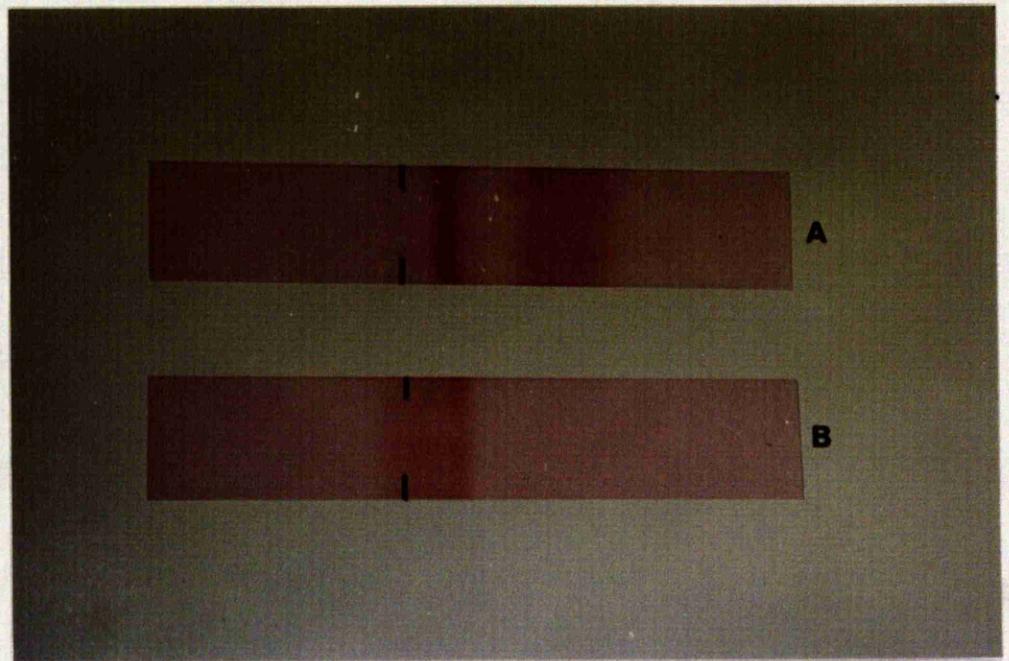
Legend for Fig. 9.4

LIPOPROTEIN PAPER ELECTROPHORESIS  
OF  $d < 1.006$  AND  $d > 1.006$  FRACTIONS

Fractions of subject no. 11.

A The strip of the  $d > 1.006$  fraction  
(load 20  $\mu$ l) shows alpha and beta lipoprotein  
bands.

B The strip of the  $d < 1.006$  fraction  
(load 40  $\mu$ l) shows a band of mobility close  
to that of the beta band in the  $d > 1.006$   
fraction.



Legend for Fig. 9.5

IMMUNOELECTROPHORESIS IN AGAROSE GEL OF LOW  
DENSITY LIPOPROTEIN FRACTION SHOWING ALPHA  
LIPOPROTEIN PRECIPITIN ARC OF NORMAL MOBILITY

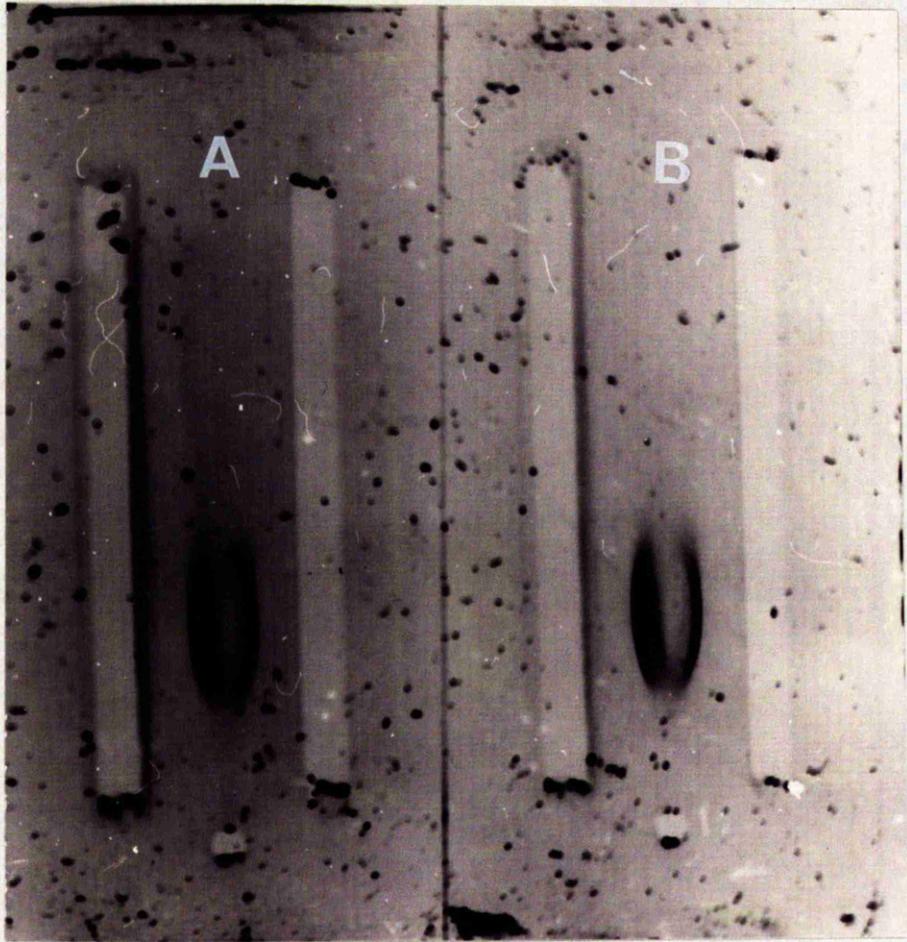
Fractions of A subject no. 11,  
B subject no. 1.

The antiserum in each left-hand trough  
was 124A with anti-alpha and anti-beta lipo-  
protein reactivity.

The antiserum in each right-hand trough  
was the monovalent anti-beta lipoprotein  
antiserum 360.

In A the low density fraction was reacted  
with antiserum 124A to produce a normal beta  
lipoprotein precipitin arc, and a faint pre-  
cipitin arc of normal  $\alpha_1$  mobility. This  
can be contrasted with the fraction in B  
which has produced a normal beta lipoprotein  
arc only.

The additional precipitin arc in A has  
not been produced with antiserum 360.



Legend for Fig. 9.6

IMMUNODIFFUSION OF LOW DENSITY LIPOPROTEIN  
FRACTION IN OUCHTERLONY PLATE SHOWING DIS-  
APPEARANCE OF ADDITIONAL PRECIPITIN LINE  
FOLLOWING REACTION WITH ANTISERUM WITH ANTI-  
ALPHA AND ANTI-BETA LIPOPROTEIN REACTIVITY  
BUT NOT WITH MONOSPECIFIC ANTI-BETA LIPO-  
PROTEIN ANTISERUM

Fraction of subject no. 11.

The centre well was loaded with antiserum  
alpha LpT<sub>1</sub> with anti-alpha and anti-beta lipo-  
protein, and anti-albumen reactivity.

The peripheral wells were loaded with  
samples of

1 and 2 - 0.10 ml low density lipoprotein frac-  
tion respectively diluted with 0.15  
ml and 0.20 ml of saline.

3 - beta lipoprotein from a normal  
subject.

4 - supernate of 0.10 ml low density  
lipoprotein fraction following  
reaction with 0.20 ml Hyland anti-  
beta lipoprotein antisera.

5 and 6/

5 and 6 - supernate of 0.10 ml low density lipoprotein fraction following reaction with 0.15 ml and 0.20 ml of antiserum 124 respectively.

7 and 8 - supernate of 0.10 ml low density lipoprotein fraction following reaction with 0.15 ml and 0.20 ml of antiserum 360.

1 shows the least diluted fraction to contain 2 immunoprecipitin lines. In 2 the fraction has been diluted so that the lines are not evident. 7 shows that the fraction which had been reacted with a volume, equal to that of the saline in 1, of the monospecific anti-beta lipoprotein antiserum 360, contains the inner line only, the outer line having been removed. The excess of antiserum 360 in 8 has reacted with the low density lipoprotein in 1 to produce a precipitin line which gives a reaction of identity with the outermost of the/

the 2 lines from 1, establishing this outer line to be due to beta lipoprotein. 5 shows both lines to have been removed following reaction with antiserum 124 with anti-alpha and anti-beta lipoprotein, and anti-albumen reactivity. The precipitin line between 4 and 5 is due to the excess of antiserum 124 reacting with an antigen derived from the material loaded in 4. This line gives a reaction of identity with a faint line between 4 and the centre well.

An identical picture is produced when other antisera with no anti-albumen reactivity were used to load the centre well.

The 2 precipitin lines in 1 may be contrasted with those in the low density lipoprotein fraction of subject no. 14 in Fig 4.15. There the additional line is peripheral to the beta lipoprotein precipitin line. Here it is internal to it.

