Section of Experimental Medicine and Therapeutics

President—G. A. H. BUTTLE, O.B.E., M.A., M.R.C.S., L.R.C.P.

[*May* 11, 1954]

DISCUSSION: THE SERUM PROTEINS

Dr. F. V. Flynn (Department of Clinical Pathology, University College Hospital, London):

Electrophoretic Patterns of the Serum Proteins in Health and Disease

The term electrophoresis refers to the migration of charged particles in an electric field. When serum protein is exposed to the influence of an electric field, under certain specific conditions, the proteins fractionate into groups of differing mobility—each group migrating towards one of the electrodes at a different velocity. The number of fractions that can be distinguished depends particularly on the species of animal from which the serum is derived and on the pH and ionic concentration of the suspending medium.

For the large majority of workers electrophoretic analysis of the proteins has remained until recently an unobtainable luxury, as the apparatus required for the classical Tiselius procedure is too costly, bulky and complex. The advent of the method of paper electrophoresis, introduced by Wieland and Fischer (1948)[1], Durrum (1950)[1], and Cremer and Tiselius (1950)[1], has entirely changed this state of affairs by placing electrophoretic analysis within the scope of even small laboratories.

Although introduced but a few years ago, paper electrophoresis now has quite a considerable literature of its own; by the middle of 1953 there were some 70 papers dealing with the subject, but nearly all of these have been concerned with method. A few papers have been mainly devoted to interpretation of results and include those of Brante (1952), Slater and Kunkel (1953), Griffiths and Brews (1953), Squire (1953), Antonini and Piva (1953), and Paton *et al.* (1954).

In the department of Clinical Pathology at University College Hospital, in collaboration with Mr. P. de Mayo, Mr. A. J. Cummings and Dr. Ann Warrick, I have examined many hundreds of sera by paper electrophoresis, and examples of important changes found in the electrophoretic pattern in health and disease are illustrated in this communication. The results obtained by paper electrophoresis can be put on a quantitative basis, but here results obtained by a qualitative technique are shown. In practice we have found that adequate information for routine clinical work can be obtained by simple visual assessment of the paper strip, provided the technique is properly standardized. The importance of studying the form of the protein spectrum cannot be over-emphasized, for information of diagnostic significance may be present there which is not conveyed in purely quantitative data.

METHOD

We have continued to use a method previously described (Flynn and de Mayo, 1951). A strip of Whatman No. 1 filter paper is supported in a special tank with its ends dipping into electrode compartments which contain a large volume of a barbiturate buffer of pH 8·6 and ionic strength 0·1. The paper is allowed to become saturated by capillarity and when the system is in equilibrium 15 microlitres of fresh non-hæmolysed serum is evenly applied in a thin line at the apex of the paper. A direct current at a potential of 110 volts is then applied for about sixteen hours. This causes the negatively charged protein particles to migrate towards the positive pole and the fractions to separate. The separated protein fractions are fixed to the paper by heat coagulation and subsequently stained by immersion in a solution of Naphthalene Black, a protein-staining dye. Excess dye is then washed off the paper.

The intensity of the colour of the stained protein bands reflects the concentration of the individual protein fractions.

THE ELECTROPHORETIC PATTERN OF THE SERUM PROTEINS IN HEALTH

(1) *The pattern in the normal adult.*—Fig. A, 1–3 shows the pattern obtained with serum and plasma. The serum shows 5 bands and the plasma an additional fibrinogen band, which is situated at the

point where the plasma was originally applied to the paper, in a position between the γ and β globulins. As this region of the electrophoretic spectrum is often of great importance from the point of view of diagnosis, we use serum in preference to plasma for routine analyses. In the absence of a fibrinogen band interpretation is much easier.

In the normal adult pattern it should be noted that albumin forms the densest band, and also—taking both colour intensity and band width into consideration—that the γ globulin band retains slightly more dye than the β globulin, which in its turn retains slightly more dye than the α_2 globulin. The α_1 globulin is normally only just discernible as a distinct band. The normal range of concentration for the various protein fractions is fairly wide, especially for the γ and α_1 fractions and this must be borne in mind when interpreting patterns from patients.

(2) *The pattern in normal infants.*—We have studied the pattern in a small number of normal infants, and typical results are shown in Fig. B, 1–3. The pattern in infancy differs somewhat from that found in the normal adult and varies significantly with age. The α_2 globulin concentration is high in infancy, increasing markedly shortly after birth, and it falls steadily with increasing age. The β globulin sometimes shows similar changes. The γ globulin concentration at birth is a little higher in the fœtal than maternal blood, but it falls thereafter to reach its lowest level between two and six months. The normal adult level is reached by 5–11 years.

(3) *The pattern in pregnancy.*—In pregnancy the pattern shows a significant variation (Fig. C, 1–3). The serum albumin concentration falls during the second three months and remains low until delivery. The α_1, α_2 and β globulins begin to increase during the second trimester and the changes become marked in the last three months. The γ globulin concentration falls slightly as the other globulin fractions increase. In toxæmia we have found that these changes are accentuated. The pattern returns to normal by two to three months postpartum.

The Electrophoretic Pattern of the Serum Proteins in Pathological States

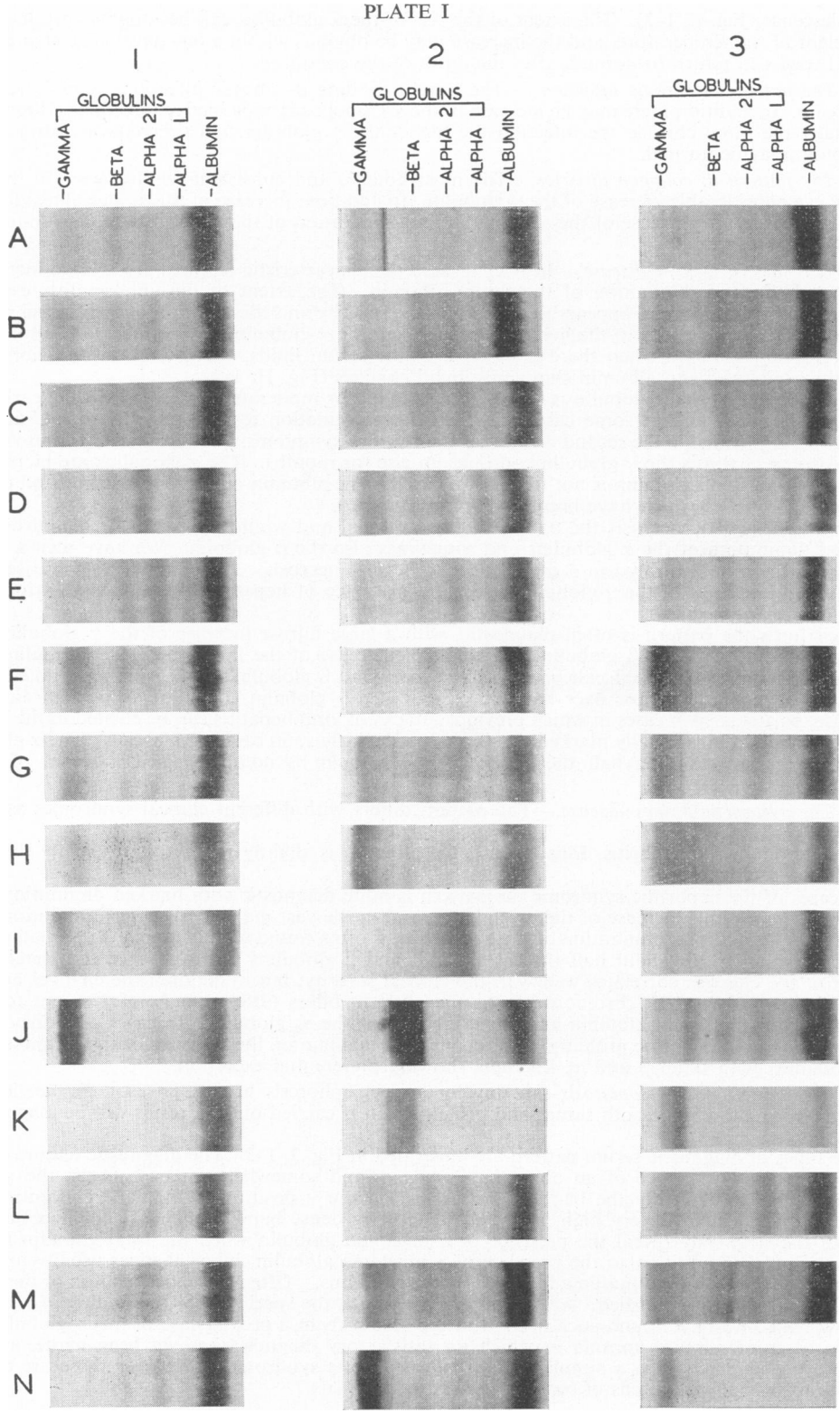
(1) *The pattern in non-bacterial tissue injury.*—In Fig. D, 1–3 the changes we have found in a few cases are shown. When tissue necrosis occurs increase of the α globulins may often be observed, and the increase may be marked within a few days. Acute hepatic necrosis, however, appears to provide an exception to this finding.

(2) *The pattern in acute infections.*—The characteristic findings in acute infections are increase of the α globulins during the acute stage, and increase of the γ globulin in the late stage and during

Key to Plate I

A.	Normal adult		
	1. Serum	2. Plasma	3. Serum
B.	Normal infant		
	1. Newborn	2. 8 months	3. 14 months
C.	Pregnancy		
	1. Early	2. Middle	3. Late
D.	Non-bacterial tissue injury		
	1. Coronary thrombosis	2. Fracture of femur	3. Irradiation
E.	Acute infection		
	1. Rheumatic fever	2. Lobar pneumonia	3. Empyema
F.	Chronic infection		
	1. Subacute bacterial endocarditis	2. Pulmonary tuberculosis	3. Kala-azar
G.	Collagen disease		
	1. Disseminated lupus erythematosus	2. Polyarteritis nodosa	3. Rheumatoid arthritis
H.	Liver disease		
	1. Infective hepatitis	2. Acute hepatic necrosis	3. Cirrhosis
I.	Kidney disease		
	1. Acute nephritis	2. Nephrotic syndrome	3. Chronic nephritis
J.	Multiple myeloma		
	1. γ-myeloma	2. β-myeloma	3. M-myeloma
K.	Urine patterns		
	1. Acute nephritis	2. Multiple myeloma	3. Multiple myeloma
L.	Malignant disease		
	1. Carcinoma of bronchus	2. Hodgkin's disease	3. Carcinoma of thyroid
M.	Miscellaneous conditions		
	1. Cushing's syndrome	2. Sarcoidosis	3. Primary xanthomatosis
N.	Rare protein disorders		
	1. Agammaglobulinæmia	2. Macroglobulinæmia	3. Cryoglobulinæmia

PLATE I


1
2
3
GLOBULINS
GAMMA
BETA
ALPHA 2
ALPHA 1
ALBUMIN
A
B
C
D
E
F
G
H
I
J
K
L
M
N

convalescence (Fig. E, 1–3). The extent of the rise of the α globulins can be roughly correlated with the height of the temperature, and the increase may be obvious within a few days. The changes take several weeks to return to normal, after the onset of the condition.

(3) *The pattern in chronic infections.*—The notable finding in chronic infections is increase of the γ globulin. In addition there may be increase of the α globulins in more active infections (Fig. F, 1–3). Generally the more chronic the infection the higher the γ globulin. In tuberculosis, however, the γ globulin may be normal.

(4) *The pattern in collagen diseases.*—The most notable and consistent feature, even in the early stages, is a considerable increase of the γ globulin. In addition, increase of the α_2 globulin is found in almost all cases and increase of the α_1 globulin and diminution of the albumin are often found (Fig. G, 1–3).

(5) *The pattern in liver disease.*—In liver disease the characteristic features are a large increase of the γ globulin and diminution of the serum albumin. The extent of the abnormality, especially diminution of the albumin, appears to depend on the severity and the duration of the disease process, but even with local lesions in the liver, e.g. metastases, the γ globulin may be increased. Sometimes the β globulin is raised when there is increase of the serum lipids, but usually the fractions other than the γ globulin and albumin show only small changes (Fig. H, 1–3).

In viral hepatitis the γ globulin is usually only slightly or moderately increased; in the early stage it may be normal and in some cases with negative flocculation tests it remains so. Usually the γ globulin is maximal in the second week after the onset of symptoms, but in cases going on to chronic hepatitis and cirrhosis the γ globulin continues to rise for months. The α globulins are increased in most cases but the β globulin is not frequently raised. The albumin only shows marked and obvious diminution in cases which have become chronic.

In massive hepatic necrosis the pattern is very unusual and would appear to be diagnostic, with marked diminution of the α globulins and sometimes also the β globulin. We have seen a pattern similar to the one illustrated in 3 cases of acute hepatic necrosis. Presumably the decrease of the albumin and increase of the γ globulin reflect the existence of hepatitis for some time prior to the onset of massive necrosis.

In cirrhosis the pattern is often diagnostic, with a large diffuse increase of the γ globulin which tends to link up with the β globulin as a result of increase of the faster moving γ globulins. This leads to absence of the usual pale area between the γ and β globulin bands. We have noted this in all types of cirrhosis in just over half the cases. The γ globulin sometimes shows an enormous increase, particularly in cases in which previous attacks of viral hepatitis can be elicited in the history. The serum albumin is usually markedly reduced, particularly so in cases with ascites. The α globulins are increased in less than half the cases. The β globulin is notably raised in cases of biliary cirrhosis.

(6) *The pattern in kidney disease.*—The pattern differs with different clinical syndromes as shown in Fig. I, 1–3.

In acute glomerulonephritis, Ellis Type I, the albumin is slightly reduced and usually the γ and α globulins are increased.

In cases of the nephrotic syndrome the pattern is quite diagnostic with marked diminution of the albumin, tremendous increase of the α_2 globulin, and in the vast majority of cases diminution of the γ globulin. In addition diminution of the α_1 globulin is often found and there may be a small increase of the β globulin. In about half the cases the α_2 and β globulins fail to resolve completely. The extent of the changes correlates well with the clinical severity, but in children the changes are more gross than in adults. In chronic nephritis the pattern differs little from normal except for some diminution of the serum albumin and some increase of the α_2 globulin. In cases of kidney disease electrophoresis of the urine protein shows, in addition to albumin, the presence of significant amounts of globulins, with often β and α_1 fractions showing preferential excretion.

(7) *The pattern in myelomatosis.*—In myeloma electrophoresis has its greatest diagnostic value. Where electrophoresis of both serum and urine protein is carried out the result will be diagnostic in more than 95% of cases.

The types of diagnostic serum pattern are illustrated in Fig. J, 1–3. The diagnostic feature of these patterns is the occurrence of an abnormal *compact* band somewhere on or between the γ and β positions: most frequently the band is somewhere on the γ position. Often the concentration of the abnormal protein is very high, giving a particularly dense band, but this is not necessarily so; indeed, the strip may reveal the presence of a myeloma globulin when the total globulin figure is quite normal. In addition to the presence of a myeloma globulin, other changes usually found are diminution of the serum albumin and increase of α globulins. Often the concentration of the normal γ and β globulin is diminished. An important question is the specificity of the finding of a compact narrow band: we have found such a band in the serum from a proved case of macroglobulinæmia, in two cases of obscure anæmia in which no satisfactory diagnosis has yet been made, and as a transitory phenomenon in a simple case of the nephrotic syndrome. However these are the only exceptions in many hundreds of sera.

Examination of the urine protein, after preliminary concentration if necessary, is extremely valuable and should be carried out whether the ordinary heat test for Bence-Jones protein is positive or not. The urine pattern may be diagnostic of myeloma when the serum pattern is equivocal. Examples of diagnostic patterns are shown in Fig. K, 1–3. A predominant protein band is found on or between the γ and β positions. In most cases the predominant protein has the mobility of a γ globulin. In myeloma either other proteins are absent or, if present, are found in much smaller concentration. When an abnormal protein is found in both serum and urine the mobility is not necessarily identical.

(8) *The pattern in malignant disease.*—In nearly all the cases we have examined the α globulins are notably increased and often the albumin is diminished. In a minority the γ globulin is increased and this may be correlated with involvement of the liver. Examples of patterns we have obtained are shown in Fig. L, 1–3. In these cases the disease was fairly advanced and it may well be that in the early stages the pattern is normal. In a few cases of lymphoma the γ globulin has been found to be diminished.

(9) *The pattern in miscellaneous conditions.*—In Fig. M, 1–3, are shown patterns from an assortment of conditions in which well-marked deviation from normal is present.

In Cushing's syndrome the interesting feature is the decrease of the γ globulin. This is of particular interest in view of the effect of cortisone treatment in lowering raised levels of γ globulin in such conditions as the collagen diseases. In addition to the low γ globulin there is marked increase of the α_2 globulin. In sarcoidosis the γ globulin is notably increased in clinically active cases and there may be some increase of the α_2 globulin. Where the disease is relatively quiescent the pattern may be normal or show only an increase of the α_2 globulin. In primary xanthomatosis there is a marked increase of the faster moving β globulins. This is of interest since it is known that the β_1 globulins transport most of the serum cholesterol. We have encountered similar patterns in other conditions associated with raised serum cholesterol; in the nephrotic syndrome, however, this finding is absent.

(10) *The pattern in rare protein disorders.*—In Fig. N, 1–3, are shown patterns obtained from cases showing very rare protein changes in the blood.

In agammaglobulinæmia there is complete absence of γ globulin. This serum came from a young child who was under the care of Dr. S. Yudkin at the Whittington Hospital and was diagnosed clinically as a possible case of agammaglobulinæmia on account of repeated pyogenic infections. Probably the condition is congenital but this remains to be proved. In the case of macroglobulinæmia illustrated there is a very dense narrow band in the γ globulin indistinguishable from a myeloma band. This serum came from a proved case of macroglobulinæmia under the care of Prof. J. Waldenstrom in Sweden and 50% of the protein has been shown by Pedersen to be of high molecular weight in the ultracentrifuge. In cryoglobulinæmia the isolated cryoglobulin from a case of myeloma is shown to migrate as a γ globulin.

Summary and Conclusions

The usual significance of the changes in the electrophoretic globulin fractions may be briefly summarized by stating that:

(*a*) The α globulins are increased in high fever; tissue destruction; nephrotic syndrome (α_2 only).

(*b*) The β globulin is increased in conditions associated with increase of the serum phospholipids and cholesterol; myeloma (some cases).

(*c*) The γ globulin is increased in chronic infections; liver disease; collagen diseases; myeloma (some cases).

In certain conditions the electrophoretic pattern is virtually diagnostic, but in most cases it will show only non-specific changes. The extent of these changes will reflect the severity if not the nature of the underlying cause. Our experience suggests that paper electrophoresis yields results similar to those obtained by the elaborate classical method, and that it has a definite value in the routine investigation of certain conditions, such as obscure anæmias. With increasing application of the method of paper electrophoresis one may safely predict that other specific findings will be found which will increase its usefulness to the clinician still further.

REFERENCES

Antonini, F. M., and Piva, G. (1953) *Recenti Progr. Med.*, **14**, 258.
Brante, G. (1952) *Scand. J. clin. Lab. Invest.*, **4**, 293.
Cremer, H. D., and Tiselius, A. (1950) *Biochem. Z.*, **320**, 273.
Durrum, E. L. (1950) *J. Amer. chem. Soc.*, **72**, 2943.
Flynn, F. V., and de Mayo, P. (1951) *Lancet*, ii, 235.
Griffiths, L. L., and Brews, V. A. L. (1953) *J. clin. Path.*, **6**, 187.
Paton, J. B., Robertson, G. K., and Wellby, M. L. (1954) *Med. J. Aust.*, i, 108.
Slater, R. J., and Kunkel, H. G. (1953) *J. Lab. clin. Med.*, **41**, 619.
Squire, J. R. (1953) *Brit. med. J.*, ii, 1389.
Wieland, T., and Fischer, F. (1948) *Naturwissenschaften*, **35**, 29.

Dr. J. Hardwicke (Department of Experimental Pathology, University of Birmingham):

Serum and Urinary Protein Changes in the Nephrotic Syndrome

We define the nephrotic syndrome as that manifested by any patient with persistent proteinuria associated with hypoproteinæmia, œdema, a raised serum cholesterol and the characteristic plasma changes described later.

For investigation the problem has been separated into two broad divisions: (i) The renal lesion and the ætiology of the proteinuria, (ii) The relationship of the proteinuria to the plasma changes and to the symptomatology of the condition. This paper is concerned with some of the findings under the second head.

The serum and urine protein changes in the nephrotic syndrome are characteristic. Fig. 1 shows a normal serum, separated by paper electrophoresis (Hardwicke, 1954) and Fig. 2 a serum and urine

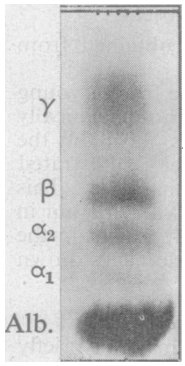


FIG. 1.—Normal serum proteins.

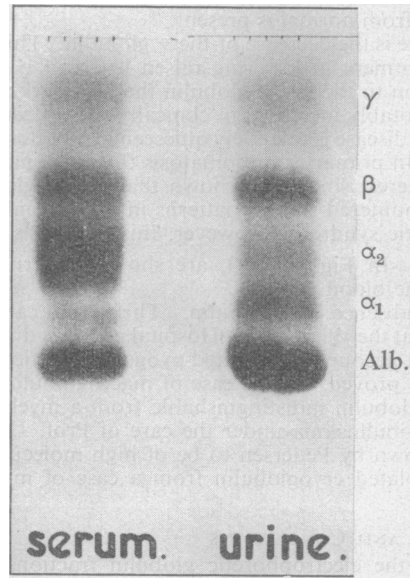


FIG. 2.—Serum and urinary proteins in the nephrotic syndrome. The serum is diluted 1/4, the urine undiluted.

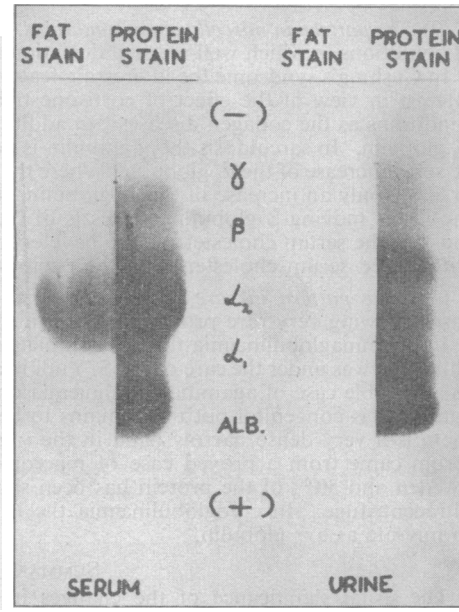


FIG. 3.—Serum and urinary proteins stained for lipid. The serum is undiluted, the urine is concentrated. × 2.

from a case of the nephrotic syndrome. In the serum the most striking changes are the low albumin and the high α_2 globulin levels. The urine shows variable proportions of albumin and the various globulin fractions in different patients, but the low molecular weight fractions (albumin, α_1 globulin and a β globulin) predominate while the high molecular weight α_2 globulin is virtually absent, in spite of the very high serum concentration. The serum lipid is also markedly raised, in association with the high serum cholesterol. This is shown in Fig. 3, in which one-half of the strip is stained with Sudan Black (Swahn, 1953). This increased lipid is associated with the slow β lipoprotein, while the fast α lipoprotein which migrates just behind the albumin, is lower than normal. The urine is strikingly free from lipid only a trace being seen in the α component. Technically this high serum lipid makes the paper method preferable to the classical U-tube electrophoretic analysis (Longsworth, *et al.*, 1939) in this type of case, since (in the U-tube) the lipid contributes to the analysis resulting in artificially low values for non-lipid containing fractions.

Fig. 4 summarizes the plasma changes, relating them all to the most striking alteration, the fall in serum albumin. It is apparent that the lower molecular weight α_1 and γ globulin fractions, which are lost in the urine, show a fall, while the high molecular weight fibrinogen and α_2 globulins are markedly raised; the combined level of β globulins which are a mixture of proteins, does not change significantly; it has, however, been shown that the β fraction lost in the urine is the low molecular weight iron-carrying globulin (Neale, 1954). These alterations in the globulin fractions only become marked when the albumin falls below 50% of normal. The changes are specific and do not appear in other forms of hypoproteinæmia such as occur with liver cirrhosis, idiopathic steatorrhœa, Whipple's disease, famine œdema or with the nutritional protein deficiency of kwashiorkor (Thompson, 1954).

Examination of the relationship between the total protein lost in the urine (gram/kg. body-weight/day) and the reduced serum albumin concentration in individual cases shows a high degree of correlation (Fig. 5), and the three cases shown conform to the same pattern. Case VII was suffering

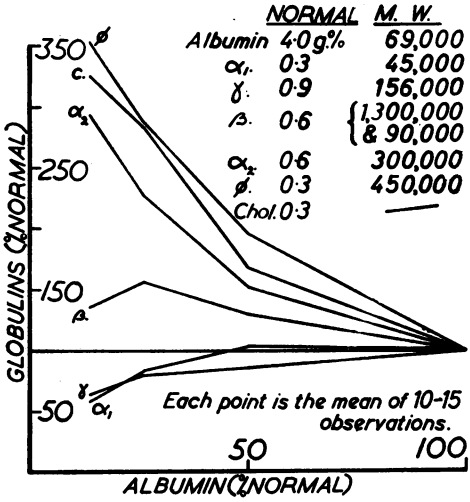


FIG. 4.—The alterations in concentration of the globulin components of plasma as the albumin level falls.

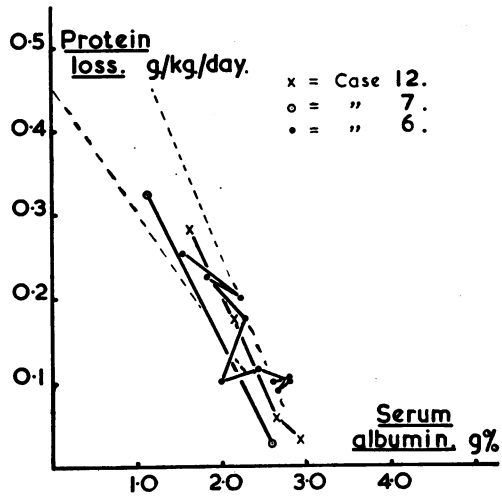


FIG. 5.—Relationship between serum albumin and daily urinary protein loss in individual cases.

from subacute nephritis in the nephrotic stage when first examined; he later progressed to chronic nephritis with isosthenuria, and as the proteinuria lessened so the serum albumin rose. Case XII was affected by constrictive pericarditis with severe proteinuria; following pericardial stripping the proteinuria diminished and finally disappeared, while the serum albumin rose steadily to normal. Case VI was a girl aged 13 with subacute nephritis who was recovering when observations were started; as the proteinuria fell the serum albumin again rose. It thus appears that in these cases, on adequate protein diet and under supervision, there is a maximum level of serum albumin compatible with a given daily protein loss.

All the cases examined, however, do not conform so satisfactorily. Fig. 6 shows the complete data, and a number of analyses show serum albumin levels disproportionately low for the protein loss; we believe that the most likely explanation is that the synthesis of protein is defective in these cases, either:

(1) Due to whole protein deprivation in the diet, or

(2) Due to specific metabolite deficiencies or metabolic disorder.

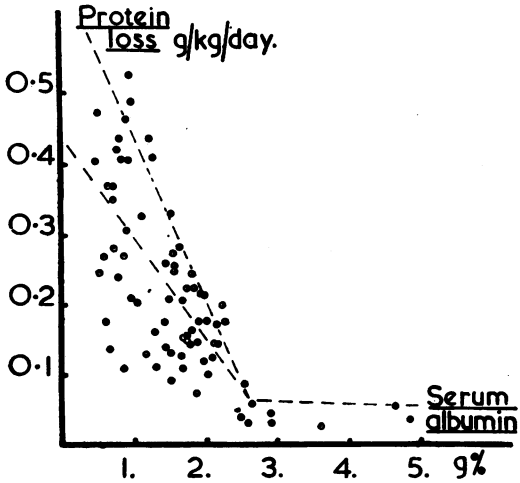


FIG. 6.—Relationship between serum albumin and daily urinary protein loss.

In favour of (1) is the observation that high protein feeding alone will, in many cases, raise the serum albumin level to the highest value usually seen in association with the daily protein loss. As the serum albumin rises, however, an increase in proteinuria must also occur; this is the result of the renal lesion already present (Squire, 1953), and does not indicate further damage; we do not therefore believe it is detrimental to the patient. The rise in serum level may be sufficient to induce a diuresis and to relieve the most distressing symptoms of the nephrotic syndrome.

In this series the nephrotic syndrome has appeared when the serum albumin was 1·0–2·0 grams % (25–50% of normal) this level being found with a protein loss of 0·15–0·3 gram/kg./day or 10–20 grams/day in a man of 70 kilo. While we believe that defects in protein synthesis may aggravate the fall in serum albumin associated with the proteinuria, more sensitive techniques, such as

the use of radioactive isotopes (Spector, 1954) will be required to supply the final answers, both as to the maximum protein synthesis possible on adequate diets, and as to the possible presence of specific deficiencies or disorders.

In conclusion, the nephrotic syndrome apparently arises in any case of proteinuria of sufficient severity (more than 0·2 gram/kg./day) and we have seen it occur: (i) In the course of progressive subacute nephritis with hypertension and microscopic hæmaturia. (ii) In a group of cases associated either with congestive cardiac failure, renal vein thrombosis or constrictive pericarditis. (iii) In a number of patients in whom no renal or systemic abnormality other than proteinuria and the characteristic plasma changes could be demonstrated; these we have called *uncomplicated nephrotic syndrome*.

The greater part of this work was carried out while in receipt of a grant from the Medical Research Council. The work in this paper forms part of a programme of investigation into proteinuria, and Professor J. R. Squire and Dr. J. D. Blainey have given much encouragement and discussed the significance of results. I am indebted to the physicians of the Queen Elizabeth Hospital, Birmingham, and other local hospitals for the opportunity of studying many patients.

REFERENCES

HARDWICKE, J. (1954) *Biochem. J.*, **57**, 166.
LONGSWORTH, L. G., SHEDLOVSKY, T., and MACINNES, D. A. (1939) *J. exp. Med.*, **70**, 399.
NEALE, F. C. (1954) Personal communication.
SPECTOR, W. G. (1954) *Clin. Sci.*, **13**, 1.
SQUIRE, J. R. (1953) *Brit. med. J.*, ii, 1389.
SWAHN, B. (1953) *Scand. J. clin. Lab. Invest.*, **5**, Suppl. 9.
THOMPSON, M. (1954) Personal communication.

Professor A. C. Frazer (Department of Pharmacology, University of Birmingham):

Blood Lipoproteins

This paper briefly reviews our present knowledge of the lipoproteins of human blood.

The importance of lipoprotein association in blood was first emphasized by Macheboeuf (1929), who prepared a lipoprotein complex with well-defined characteristics from horse serum. Since then a number of lipoprotein particles have been isolated by different methods. Pedersen (1945) described a lipoprotein isolated from human plasma. Gurd *et al.* (1949) isolated an α and β lipoprotein from human plasma by differential alcohol precipitation. Gofman and his colleagues (1950) separated a series of fractions with differing flotation rates by ultracentrifugation. The most interesting groups, biologically, appear to be the lipoproteins with flotation rates Sf 10/20 and Sf 30/100. The density relationships are shown in Table I.

TABLE I

Flotation rate	Density grams/c.c.
Sf 2	1·050
Sf 4	1·040
Sf 8	1·029
Sf 10	1·023
Sf 17	0·99

Sf 40,000 represents chylomicrons

The Properties of Blood Lipoproteins

The lipids and proteins are linked by various bonds to form a mixed lipoprotein molecule which, in spite of a high lipid content, displays the general characteristics of a protein. Other types of lipoprotein association may occur which give lipid characteristics to the complex; these have been termed proteolipids by Folch and Lees (1951).

The size of the lipoproteins varies. Gurd *et al.* (1949) concluded that their α lipoprotein was 300 Å long and 50 Å wide and had a molecular weight of 200,000. This corresponds in size to the smallest measurable chylomicron observed under dark-ground by Elkes *et al.* (1939). The β lipoprotein, on the other hand, was spherical, with a diameter of 185 Å and had a molecular weight of 1,300,000. It resembles the X-protein of Pedersen. The lipoproteins isolated by ultracentrifuge vary in size—the Sf 10/20 group have a mean diameter of about 250 Å.

The composition of the lipoproteins has also been studied: The α lipoprotein accounts for 3% of the plasma protein, contains 35% of lipid and 65% of protein. The β lipoprotein represents 5% of the total plasma protein and 75% of the lipid in fasting plasma. It contains 75% of lipid (2/5 phospholipid and 3/5 cholesterol) and 25% of protein. The lipoproteins isolated by ultracentrifugation at flotation rate Sf 4 contain 25% of protein, 30% of cholesterol, 45% of phospholipids and no glycerides. Glycerides begin to occur in the lipoprotein fraction of lighter density than the Sf 17 series and increase progressively, while protein, cholesterol and phospholipids decrease.

Variations in lipoproteins.—The most interesting information at present available on the variations in blood lipoprotein under differing physiological or pathological conditions is concerned with the amounts of Sf 10/20 and Sf 30/100 lipoprotein found on ultracentrifugation.

Effect of age and sex.—The blood of children contains low levels of the Sf 10/20 and Sf 30/100 groups. These groups tend to increase with age; below 40 this is more marked in the male, but after 40 there is no sex differentiation (Gofman *et al.*, 1951).

Diurnal variation.—No significant diurnal variations were observed in the Sf 10/20 lipoproteins; a small but definite diurnal variation was found in the Sf 30/100 group, related to dietary intake of fat (Chandler *et al.*, 1953).

Effect of hormones.—Sex hormones appear to alter the distribution of lipoproteins; changes are observed during pregnancy. Heparin and allied substances have a marked effect, causing an immediate decrease in the level of the Sf 30/100 particles and a delayed and less-marked decrease of the Sf 10/20 group (Chandler *et al.*, 1953; Graham *et al.*, 1951).

Choline and other lipotropic substances were thought to be ineffective in relation to the lipid deposits of atheroma, but a recent publication by Best and his colleagues (Wilgram *et al.*, 1954) suggests that this may not be the case.

Correlation with obesity.—Some correlation has been shown between the occurrence of obesity and the level of Sf 10/20 and Sf 30/100 particles (Gofman and Jones, 1952).

Correlation with atherogenesis.—There would appear to be a significant correlation between an increase in the levels of Sf 10/20 and Sf 30/100 lipoprotein particles and atherogenesis, both in experimental animals and human subjects. Improvement is claimed in patients with severe atheroma and concomitant pathological changes during periods when the levels of these lipoproteins are depressed by heparin (Graham *et al.*, 1951).

Correlation with blood lipid levels.—It has long been thought that blood cholesterol levels or the cholesterol/phospholipid ratio correlate significantly with atherogenesis. There is some indication that these blood lipid levels may be correlated with certain lipoprotein changes, but the evidence is conflicting. It has been claimed that the abnormal lipoprotein levels are no more highly correlated with atherogenesis than total blood cholesterol (Keys, 1952). The lipoprotein fractions regarded as of possible significance in atherogenesis account for about 10% of the blood cholesterol.

Functions of blood lipoproteins.—Lipoproteins and their constituent lipids—cholesterol and phospholipids—have been thought to play some part in lipid transport. Recent studies suggest that these lipids do not represent transportable lipid which is being moved from one place to another for the purposes of metabolism. The rate of utilization of these materials by the extra-hepatic tissues is slow and the main site of both production and utilization appears to be the liver (Entenman *et al.*, 1946). The levels in the blood, therefore, mainly reflect the situation in the liver. The smaller lipoproteins, including the Sf 10/20 group, may be structural components of plasma. The Sf 30/100 group contains the main transportable lipid, triglyceride esters—this group consequently shows diurnal variations.

The occurrence of lipid deposition in the blood vessels has been regarded by some as a sort of "super-saturation" of the blood plasma with lipid. The appearance of abnormal levels of Sf 10/20 lipoproteins was not incompatible with this view. Recent work, however, indicates a different trend of thought. The turnover of lipids in the vessel wall is different in the atheromatous as compared with the normal vessel. The question, therefore, arises whether the changes observed in the blood lipoproteins may be part of a wider abnormality of lipid metabolism affecting the blood vessel walls and perhaps other tissues (Biggs and Colman, 1953).

It would be unwise to attempt to draw any conclusions at present with regard to the precise significance of changes in the blood lipoprotein pattern. It may be accepted, however, that changes in the blood lipoproteins can be significantly correlated with atherogenesis and that these changes are reversible. If it is true that the blood changes are only indicative of a more generalized abnormality of lipid metabolism, it is not impossible that certain tissue changes may also prove to be reversible.

REFERENCES

BIGGS, M. W., and COLMAN, D. (1953) *Circulation*, **7**, 393.
CHANDLER, H. L., LOWRY, E. V., POTEE, K. G., and MANN, G. V. (1953) *Circulation*, **8**, 723.
ELKES, J., FRAZER, A. C., and STEWART, H. C. (1939) *J. Physiol.*, **95**, 68.
ENTENMAN, C., CHAIKOFF, I. L., and ZILVERSMIT, D. B. (1946) *J. biol. Chem.*, **166**, 15.
FOLCH, J., and LEES, M. (1951) *J. biol. Chem.*, **191**, 807.
GOFMAN, J. W., and JONES, H. B. (1952) *Circulation*, **5**, 514.
——, ——, LINDGREN, F. T., LYON, T. P., ELLIOTT, H. A., and STRISOWER, B. (1950) *Circulation*, **2**, 161.
——, LINDGREN, F. T., JONES, H. B., LYON, T. P., and STRISOWER, B. (1951) *J. Geront.*, **6**, 105.
GRAHAM, D. M., LYON, T. P., GOFMAN, J. W., JONES, H. B., YANKLEY, A., SIMONTON, J., and WHITE, S. (1951) *Circulation*, **4**, 666.
GURD, F. R. N., ONCLEY, J. L., EDSALL, J. T., and COHN, E. J. (1949) *Disc. Faraday Soc.*, **6**, 70.
KEYS, A. (1952) *Circulation*, **5**, 115.
MACHEBOEUF, M. (1929) *Bull. Soc. Chim. biol., Paris*, **11**, 268, 483.
PEDERSEN, K. O. (1945) Ultracentrifugal studies on serum and serum fractions. Uppsala.
WILGRAM, G. F., HARTOFT, W. S., and BEST, C. H. (1954) *Brit. med. J.*, ii, 1.

Professor N. H. Martin (St. George's Hospital Medical School, London):

Experience with the Transfusion of Human Albumin into Patients with Established Liver Damage

Since 1950, when Kekwick and Mackay (1954) first produced pure human albumin by their ether fractionation method in quantities sufficient to make limited clinical trial possible, we have, in the course of a more general study, transfused and followed in the succeeding years 17 patients suffering from progressive liver failure. In this note two points only, out of the mass of accumulated data, are discussed. A general discussion of the complications experienced with the albumin has already been published (Martin, 1954).

Eckhardt *et al.* (1948) have shown that albumin whether hydrolysed or whole, given orally, was accompanied by a prompt increase in the daily output of urinary nitrogen.

In contradistinction, intravenous administration of human albumin into normal persons did not result in an immediate corresponding increase in urinary nitrogen excretion, there being a lag of about three days (Eckhardt and Davidson, 1950).

In Fig. 1 the results of balance studies on 3 patients with chronic liver damage which we have studied, illustrate the same pattern of lag as that observed in normal subjects. The patients were all at rest in bed through the whole period of observation, and had been on the protein intakes noted in the figure for at least three weeks before the metabolic studies illustrated. None of the 3 patients

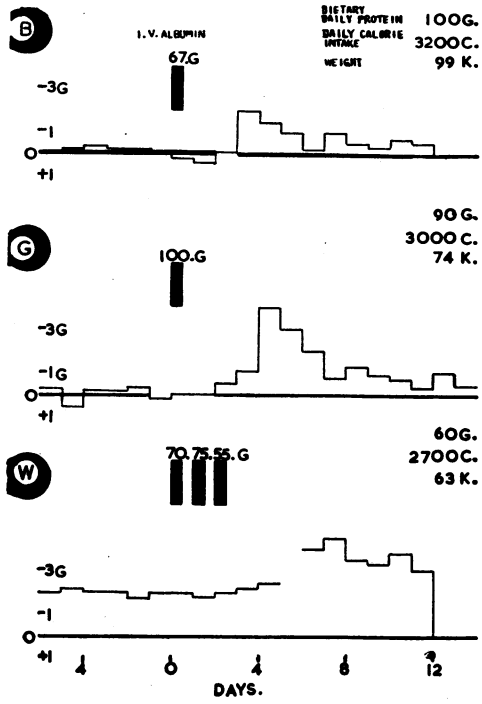


FIG. 1.—Nitrogen balance studies on three patients, B, G, and W, suffering from chronic progressive liver damage. The black transverse line at O indicates the point at which *oral* nitrogen intake was balanced by fæcal and urinary nitrogen output. The black oblongs represent the albumin transfusions each of which consisted of a 17·5% solution of salt-poor human albumin in 5% aqueous glucose solution (for sodium content ref. *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 286). The supplementary nitrogen introduced into the daily diet through the transfusion is not included in the total of the balance data. On day O each patient would be in markedly +ve nitrogen balance.

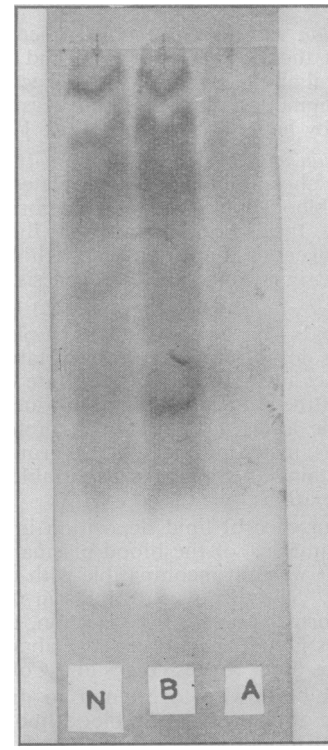


FIG. 2.—Qualitative chromatogram run in Butanol acetic acid on Whatman No. 1 filter paper. 50 μl. samples of each urine were mounted at the respective starting points. N = 8 a.m. specimen normal control. B = 8 a.m. specimen from patient R. S. prior to a transfusion of 75 grams of human albumin in five hours. A = 8 a.m. specimen from patient R. S. the morning after the transfusion was completed.

developed proteinuria during the period of study, though we have observed proteinuria in other patients receiving albumin intravenously in single doses of 100 grams and upwards.

Dent in 1947 had described briefly the increase and alteration in urinary amino-acid pattern occurring occasionally in patients with advanced liver damage. This does not occur regularly in all such patients. Nevertheless, as opportunity arose, we examined the effects of intravenous albumin on this phenomenon, especially in the "lag" period following transfusion.

Fig. 2 shows the immediate effect of intravenous albumin on the output of amino acids of a patient whose urine contained the marked increase described by Dent. As will be seen, there was an immediate and almost complete removal of amino acids from the urine which could not be explained on the grounds of a sudden excessive diuresis.

We had noticed in 3 patients suffering from extremely advanced liver damage a transient though striking improvement following single massive transfusions, and this observation was therefore of peculiar interest, hinting at the possibility that some of the free circulating amino acids may contribute to the clinical picture of hepatic coma, and that their "temporary immobilization" by albumin results in transient clinical improvement.

Walshe (1953) has made an extended study of the pattern of amino-acid excretion in liver disease and contends that the increased excretion is due to a raised level of free circulating amino acids rather than altered renal tubular reabsorption. A number of workers (Klotz, 1949; Martin, 1949) have demonstrated the extent to which albumin may interact with a variety of small molecules *in vitro*. We suggest that the alteration in urinary amino acids illustrated is due to a temporary "mopping up" of the excess of free circulating amino acids by the fresh transfused albumin. This "mopping up" would effectively, though temporarily, prevent their loss through the kidney in the urine.

This "mopping up" of amino acids is, in our experience, transient, the urinary amino acids returning to their pretransfusion level in the course of the next eight days.

Eckhardt and Davidson (1950) calculated that of 450 grams of human albumin given discontinuously to a normal human volunteer over a period of six days, 40·7% could be accounted for by the increase in total circulating albumin. This was in line with the earlier observation of Janeway and his associates (1944).

During the twenty-one days from the start of the albumin transfusions, 56% of the albumin transfused could be accounted for in the urine. Of this loss 43% was excreted in the fifteen days immediately after cessation of the transfusion and only 12–13% in the six days of the actual transfusion. It follows, therefore, that

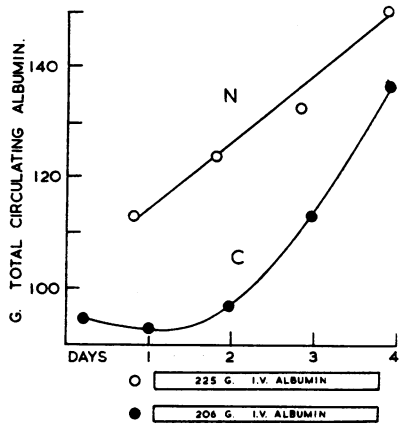


FIG. 3.—Alteration in circulating albumin levels over a three-day period. Grams total circulating albumin calculated from plasma volume and serum protein studies. N = normal volunteer. C = Patient with advanced progressive liver damage. Crown copyright: reproduced by permission of H.M. Stationery Office.

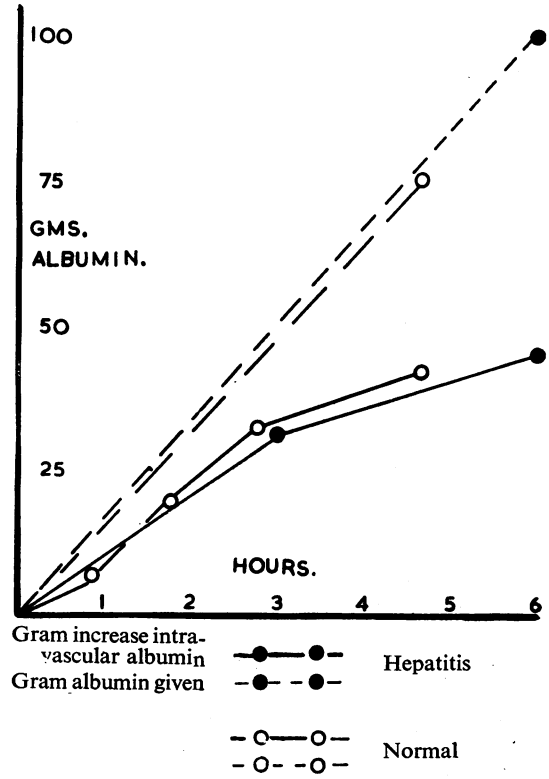


FIG. 4.—Albumin retained in the vascular compartment during the progress of a single transfusion of albumin maintained at a steady rate. Albumin concentration in transfusing fluid. 17·5 grams per 100 ml. 5% glucose in water.

during the six-day period there must have been some sort of extravascular deviation of the order of 50%.

Fig. 3 compares the extravascular deviation in one of our cirrhotics who was given 205 grams of albumin in three days. Her serum albumin level before transfusion was 2·3 grams/100 ml. compared with an albumin level of 3·6 grams/100 ml. in a normal control. At the completion of the transfusion her serum albumin level had risen to 3·6 grams/100 ml. compared with the level in the control of 4·7 grams/100 ml. over a period in which daily doses of intravenous albumin were given of a magnitude comparable with Eckhardt and Davidson.

Superficial inspection suggests that the extravascular deviation is greater in a person with chronic liver damage than in a healthy adult of equivalent weight. The patient illustrated in Fig. 3 had clinically demonstrable ascites shortly after the transfusion, and we cannot be absolutely certain that there was no free fluid in the abdomen at the time of the transfusion.

At the next opportunity we checked the extravascular deviation in a patient with proved progressive hepatic damage but with no ascites or œdema, whose weight had been constant over several weeks. Before transfusion the circulating albumin level was 3·1 grams/100 ml. Fig. 4 shows the effect of a transfusion of 100 grams of albumin in six hours compared with a normal control, having a circulating albumin level of 3·8 grams/100 ml. before the transfusion, receiving 75 grams of albumin at a comparable rate of flow. The albumin concentration in the transfused fluid was 17·5 grams per 100 ml. in each instance. Both subjects were at rest in bed and in positive nitrogen balance. The graph suggests that the rate of extravascular albumin deviations differs very little in the patient with liver damage from the normal person. Moreover it appears that with infusions larger than 50 grams in three hours the extravascular deviations increase sharply, corresponding to a transfusion rate of about 0·20–0·25 gram albumin per kilo per hour.

The feeling of heaviness and headache, the precursor of frank clinical over-loading (Martin, 1954), was beginning to be felt by the control subject at three and a half hours and was well established by four hours and forty minutes when the transfusion was stopped. It appears, therefore, to correspond with the second period in which albumin is being forced out into the extravascular space.

Table I gives an approximate balance sheet disposal of 100 grams of infused albumin over the succeeding seventy-two hours from our own data and from that in the literature.

TABLE I.—IN THE FIRST 72 HOURS AFTER THE INTRAVENOUS ADMINISTRATION OF 100 GRAMS OF ALBUMIN

25 to 50 grams	accounted for in the VASCULAR space
0 to 15 grams	in the URINE
35 to 75 grams	being distributed through the LYMPHATICS and the EXTRAVASCULAR areas of the BODY

CONCLUSION

We have selected these two observations to illustrate two aspects of the effects of intravenous albumin therapy whose importance we think is not adequately stressed.

The first that the distribution of albumin is not confined to the vascular compartment. We never did believe this but we wished to show how both in health and disease there can be relatively rapid transfer of albumin to and from the vascular, to the lymphatic, to the extracellular space and perhaps to the cells themselves.

The second springs from the proved ability (Klotz, 1949; Martin, 1949) of albumin, a highly charged ampholyte, to interact with small molecules. This capacity gives to albumin (in so far as it may temporarily attract to its reactive surface a wide variety of small molecules) an important role in the control of the rate of disposal of these small molecules.

ACKNOWLEDGMENTS

My thanks are due to the Physicians of St. George's Hospital for giving me the opportunity to carry out these trials and to the Hospital Endowment Fund for financial assistance during the progress of the analytical work in the department.

REFERENCES

DENT, C. E. (1947) In: Sixth Conference on Liver Injury. Editor: F. W. Hoffbauer. Josiah Macy, Jr., Foundation, New York; p. 53.

ECKHARDT, R. D., and DAVIDSON, C. S. (1950) In: Plasma Proteins. Editor: J. B. Youmans. Symposia on Nutrition of the Robert Gould Research Foundation, Springfield; **2**, 275.

——, LEWIS, J. K., MURPHY, T. L., BATCHELOR, W. K., and DAVIDSON, C. S. (1948) *J. clin. Invest.*, **22**, 119.

JANEWAY, C. H., GIBSON, S. T., WOODRUFF, L. M., HEYL, J. T., BAILEY, O. T., and NEWHOUSER, L. R. (1944) *J. clin. Invest.*, **22**, 465.

KEKWICK, R. A., and MACKAY, M. E. (1954) *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 286, p. 1.

KLOTZ, I. M. (1949) *Cold Spr. Harb. Symp. quant. Biol.*, **14**, 97.

MARTIN, N. H. (1949) *J. Amer. chem. Soc.*, **71**, 1230.

—— (1954) *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 286, p. 60.

WALSHE, J. M. (1953) *Quart. J. Med.*, N.S. **22**, 483.