THE VALUE OF PAPER ELECTROPHORESIS OF SERUM PROTEINS IN DIAGNOSIS OF ASCITES

BY

H. E. M. KAY, M.D., M.R.C.P.

From the Department of Pathology, St. Thomas's Hospital Medical School, London

Changes in the serum electrophoretic patterns, reflecting, as they frequently do, the stage or intensity of a disease rather than indicating its exact cause, are seldom likely to be of great value in a purely diagnostic capacity. Moreover, the absence of literature concerned with clinical application as opposed to method and refinements of electrophoresis is a sign that there is some doubt of its usefulness in this field, and it seems that the wave of initial enthusiasm that greeted the introduction of the paper method is already fast receding. In this context it may be profitable to take a single clinical problem which arises from time to time and which demonstrates the possibilities as well as the limitations of electrophoresis.

One such problem is the patient whose predominant manifestation of disease is ascites. This is most often due to cirrhosis of the liver or peritoneal metastases of carcinoma; less often, a malignant tumour, usually of the liver but without direct peritoneal involvement, is found; while in other cases constrictive pericarditis, cardiac failure, or subacute nephritis may be the primary condition (although in the last-named disorder the diagnosis is seldom in doubt and in cardiac failure ascites is usually an insignificant part of the total syndrome). The distinction between hepatic and malignant disease, however, may be a matter of some difficulty, for, even if other signs and symptoms are present, they are often of a vague nature or are common to both (hepatomegaly, anorexia, haematemesis, etc.).

In these circumstances serum electrophoresis may be a most valuable guide, since the changes in each condition are quite distinct and, as is shown, reasonably constant. Briefly, in chronic liver disease a variable increase of γ -globulin accompanies low or low normal α -globulin levels, while in malignant disease low, normal, or slightly increased γ -globulin levels are overshadowed by a well-marked α -globulinaemia. In constrictive pericarditis and congestive cardiac failure insufficient evidence is available for generalization, but from limited data (Luetscher, 1941, and personal observations), and on theoretical grounds, either a normal pattern or a moderate increase of both α - and γ -globulin might be expected. Intra-hepatic secondaries seem, for reasons which will be considered later, to give diverse patterns, while subacute nephritis produces a well-known pattern. In this a prominent band of α -2 lipoprotein is contrasted with a considerable reduction of all other protein fractions. This may resemble the pattern of some forms of malignant disease; the distinction is discussed below.

Methods

Electrophoresis of serum was performed by the method of Flynn and de Mayo (1951)—10 to 20 μ l. of serum (20 to 30 μ l. of ascitic fluid) being applied to No. 1 Whatman paper. Azocarmine Bx (Turba and Enenkel, 1950) was preferred as a protein stain, since there is less background staining than with naphthalene black, and, unlike bromphenol blue, albumin and globulins take up the stain equally well (that

is, in proportion to their nitrogen content). After drying, the paper was cut up into five pieces (corresponding to the protein bands) and the dye was eluted in 5 ml. N/10 NaOH. The optical density of the eluate was determined in a Hilger absorptiometer and the relative concentrations of the protein fractions were determined. Total serum protein was measured by the micro-Kjeldahl method.

Results

Thirty-eight consecutive* cases of ascites have been studied by paper electrophoresis of serum and, in a few cases, of ascitic fluid. In general, serum is preferable, since its higher protein content produces clearer, denser bands and it is usually more easily available. Ascitic fluid, it appears, resembles diluted serum but with relatively more albumin and with globulin concentrations which are less constantly related to the cause of the ascites. The subject has been investigated and discussed by Luetscher (1941). A summary of the serum analyses in the present series is shown in Table I, and from these the most significant results have been plotted in Figs. 1 and 2.

Carcinomatosis Peritonei and Hepatic Cirrhosis

These two major groups of cases are so well defined and divided from each other that comment at this stage is superfluous. However, two cases—one from each group—in which electrophoretic analyses seemed to be partially in conflict with clinical and other laboratory evidence may be worth quoting.

FIG. 1

FIG. 2.—Key as Fig. 1.

*Omitting cases of cardiac failure and one case in which the diagnosis is uncertain.

TABLE I.—Serum Protein Analyses

Diagnosis	Total	Albumin	Globulin			
			α -1	α -2	β	γ
Carcinomatosis peritonei (15 cases)	6.07 $\pm$ 0.79	2.34 $\pm$ 0.65	0.56 $\pm$ 0.14	1.10 $\pm$ 0.25	0.93 $\pm$ 0.20	1.14 $\pm$ 0.53
Cirrhosis of liver (16 cases)	6.58 $\pm$ 0.72	2.26 $\pm$ 0.48	0.36 $\pm$ 0.09	0.50 $\pm$ 0.15	0.81 $\pm$ 0.20	2.65 $\pm$ 1.13
Miscellaneous						
G.P.	5.00	1.70	0.36	0.65	1.20	1.07
M.W.	5.22	1.96	0.65	0.42	0.90	1.29
F.F.	7.60	2.70	0.67	0.76	1.27	2.20
Pericarditis:						
1	6.62	3.94	0.36	0.50	0.87	0.96
2	6.50	3.66	0.34	0.60	0.82	1.08
3	7.20					
? Subacute nephritis S.H.	4.34	1.02	0.29	1.10	1.06	0.87

Serum protein is expressed as g./100 ml. serum with standard deviations of initial group.

Case 1.—An obese woman of 65 complained of progressive abdominal distension of one month's duration. This was accompanied by a vague feeling of ill-health together with occasional vomiting, and for one week a slight pain in the right side of the abdomen. A mild attack of jaundice at the age of 12 was possibly relevant. Examination revealed a grossly distended abdomen with oedema of the legs and varicose veins. The only other abnormal findings were a blood pressure of 230/120 and doubtful icterus. Laboratory investigations: Hb 79% (Haldane); E.S.R., 35 mm. in one hour (Westergren); blood urea, 23 mg. per 100 ml.; serum protein, 6.2 g.% (albumin 2.1 g., globulin 4.1 g.—sodium sulphate precipitation); alkaline phosphatase, 12 King-Armstrong units; thymol turbidity, 3 units; icteric index, 17 units. Paracentesis produced 14½ pints (8.2 litres) of clear yellow fluid which was thought to contain fragments of a columnar-celled carcinoma. Electrophoresis, however (Fig. 3, A), showed that most of the excess serum globulin was γ -globulin (albumin, 2.21 g.; α -1, 0.35 g.; α -2, 0.62 g.; β , 0.75 g.; γ , 2.27 g.), and at necropsy, several months later, cirrhosis of the liver was found.

FIG. 3.—Results of serum electrophoresis in five cases.

Case 2.—A woman of 53 complained of intermittent abdominal pain for three days with swelling of the abdomen for two weeks and a cough for one year. An attack of jaundice at the age of 19 had lasted for six weeks. On examination she was pale and possibly slightly icteric, with spider naevi on the face and neck. The only physical signs were distension of the abdomen and shifting dullness. Urine: slight excess of urobilinogen. Blood: Hb, 92% (Haldane); blood urea, 42 mg. per 100 ml.; serum protein,

6.6 g.% (albumin 3.4 g., globulin 3.2 g.—sodium sulphate precipitation); thymol turbidity 2 units; alkaline phosphatase, 13 King-Armstrong units. Faeces showed no occult blood. Paracentesis produced 13½ pints (7.7 litres) of fluid, which contained lymphocytes and endothelial cells only. At this stage a presumptive diagnosis of hepatic fibrosis was made, but electrophoresis (Fig. 3, B) seemed to be against this (albumin, 2.12 g.; α -1, 0.61 g.; α -2, 1.37 g.; β , 1.10 g.; γ , 1.37 g.). Later, after a second paracentesis, a mass was felt in the left iliac fossa, and subsequent laparotomy revealed a carcinoma of the ovary with diffuse peritoneal metastases.

Miscellaneous

In three cases carcinoma of different sites had, without peritoneal metastases, led to the formation of ascites. As their protein analyses differ from both the main groups (see Table I and Figs. 1 and 2) they are worth consideration in some detail.

The first, G.P., with obstructive jaundice of three months' duration prior to the occurrence of ascites, was found at laparotomy to have a carcinoma of the head of the pancreas. It is possible that this caused some degree of portal obstruction; but the patient might also be supposed to have had some degree of biliary cirrhosis and in addition the serum albumin concentration was very low (1.70 g.%). These factors in combination were probably responsible for the ascites.

M.W., with a well-marked α -1 globulinaemia without α -2 globulinaemia, which is most unusual (Fig. 3, C)—died after a short illness marked by fever, jaundice, and hepatomegaly. At necropsy the liver tissue was found to be largely replaced by a mass of growth, judged on histological grounds to have arisen from biliary epithelium. Neither hepatic nor portal veins were thrombosed, so that the same factors were probably operative in producing ascites as in the previous case.

In F.F. a large necrotic carcinoma of the right lower lobe bronchus had infiltrated the right auricle, the diaphragm, and much of the upper part of the liver. Thrombosis of the inferior vena cava had occurred below the level of the renal veins. Microscopy of the liver showed intense centrilobular congestion and necrosis of the adjacent liver cells, but only slight fibrosis. A complicating factor was the presence of rheumatoid arthritis, and with such a combination of conditions almost any sort of protein pattern might be expected. It is perhaps surprising that with so much tissue disorganization and destruction the increase of α -globulin should be so slight. However, if α -globulins do originate in the liver (see below) it is possible that the embarrassment of hepatic function would have inhibited their formation, thus producing a pattern which most closely resembles the group of hepatic fibroses. This last case illustrates clearly the chief limitation of serum electrophoresis—namely, its incapacity in any case of a complicated nature to distinguish the pathological processes that are occurring.

Constrictive Pericarditis

Three patients were found to have constrictive pericarditis, and, whereas two of these had essentially normal protein

patterns, the third serum produced a curious "plateau" pattern from the γ to the α -2 (Fig. 3, D), which completely defies both analysis and explanation.

Subacute Nephritis

Finally, there remains one patient, S.H., in whom the diagnosis is still uncertain; but as the uncertainty may well persist, and as the case raises some points of interest, it seems worthy of quotation.

Case 3.—A woman aged 58 presented with gross oedema of the legs and ascites. Persistent and fairly heavy albuminuria was noted, accompanied by occasional granular and hyaline casts, while the serum contained a reduced protein concentration (4.34 g.%) and raised cholesterol (474–515 mg. per 100 ml.). In addition, however, several tests for occult blood in the stools were strongly positive. Intravenous Congo red was not taken up by the tissues.

The protein strip revealed a pattern that is compatible with either Type II nephritis or malignant disease. Theoretically, these should be distinguishable by determining the nature of the α -2 globulin. In nephritis this is largely lipoprotein; in conditions of tissue destruction (including amyloid disease) it is mainly glycoprotein. On staining with Sudan III and periodic-acid-Schiff the α -2 band turned out to contain polysaccharide rather than fat, which would support a diagnosis of amyloid disease or carcinomatosis. Nevertheless the course of the disease in this patient to date is much more suggestive of nephritis.

Discussion

In considering the results obtained in this investigation three main questions may be posed: How far do they agree with the results of other similar studies?; What are the basic pathological mechanisms underlying the changes described?; and, Are these changes sufficiently constant to be applicable in diagnosis?

The last question may be taken first, and can be answered in the affirmative. Although the number of cases reported is small, they are grouped closely and distinctly enough to include the overwhelming majority of any subsequent cases that might be added to them. Statistical significance is really irrelevant in this situation even though the probability and extent of overlapping in larger series might be estimated. Furthermore, it is suggested that in clinical practice even the analysis of strips is scarcely necessary. For the average case visual judgment will suffice; in the doubtful one, it is unlikely to help. The conclusion that may be drawn is that a clear distinction can be made between ascites due to peritoneal metastases and that due to intrahepatic causes. Furthermore, if taken in conjunction with other tests—especially, for example, the E.S.R. and thymol turbidity—serum electrophoresis will always be found to be of some assistance in the elucidation of peritoneal effusions.

In seeking for corroborative evidence from other sources there is a mass of information to be found on the plasma proteins both in cancer and in liver disease. Winzler (1953), for example, in a review of the former subject, refers to over 300 relevant papers, but of these only one contains comprehensive and detailed observations of electrophoretic analyses. That study (Mider *et al.*, 1950) included 258 patients with uncomplicated cancer which was classified according to the stage of the disease (localized, advanced, and late) and to a certain extent as regards the site (but not the histological type) of the growth. The relevant figures are shown in Table II, and these may be compared with the

first part of Table I. Excepting only the γ -globulin levels, there is good agreement, especially with Mider's category of late cancer. The γ -globulin discrepancy may be purely a technical one for which a number of explanations may be offered,* or it may reflect some fundamental aspect of the body's reaction to a particular sort of malignant process. Unusually high globulin levels (probably mainly γ -globulin) have been noted occasionally in malignant disease (Bing, 1940; Snapper, 1943), but, with the exception of myelomatosis, in which the tumour cells undoubtedly produce the abnormal increment, their significance is obscure. The theory of humoral resistance to invasive tumours by means of antibodies is now out of favour, but it is possible that antibody formation may be stimulated by some of the products of tumour breakdown, especially if the peritoneal cavity is acting as a large test-tube in which a variety of cellular disintegrations is occurring.

Of some interest in this connexion was Case A.L., with markedly raised serum γ -globulin (Fig. 3, E). An ovarian carcinoma with widespread peritoneal metastases was demonstrated at laparotomy, but in addition the bone marrow was found to contain some 5% of plasma cells.

Whatever may be the case with γ -globulin, there is little doubt that the presence of a large area of tissue disorganization of destruction is closely bound up with the α -globulin-aemia. The association of inflammation and tissue destruction with increase of both α -globulin bands was first noted by Longworth *et al.* (1939), and many similar observations—for example, of increases of serum protein-bound polysaccharide (most of which occurs in the α -bands)—have since been made. As yet no hypothesis has been advanced to explain the relationship. "The plasma α -globulin levels may reflect the activity of generalized processes such as tissue destruction or replacement" was as far as Winzler could go in 1953.

Synthesis of α -globulins may well occur in the liver. Experimental evidence is scarce and conflicting, but recent isotope studies have clearly demonstrated the hepatic origin of many globulins (Miller *et al.*, 1950). Furthermore, in uncomplicated liver disease in man α -globulins tend to be slightly lower than normal (Martin, 1949), although from the destructive and inflammatory nature of hepatitis one might expect the opposite. The opposite figures in the present series are around "low normal" levels, being of the same order as those of other observers.

The γ -globulin levels, too, are in line with other electrophoretic studies (Gray and Guzman Barron, 1943; Martin, 1949), the variation, with a range of 1.65–6.22 g.%, being characteristically wide. It was noted that some of the lower concentrations, although definitely abnormal, coincided with thymol turbidity readings of 3 or less (that is, within the range of normality). This finding enhances the value of direct γ -globulin estimations, although, as a rule, in chronic liver disease the thymol test is a good guide to the γ -globulin level. What actually determines the level of serum γ -globulin in these cases is still a matter for speculation. Bjørneboe (1950) has suggested antibody formation, and in the case of very high levels—for example, 6–8 g.%—this seems the most probable explanation; moreover, there appears to be some slight correlation between the intensity of the inflammatory reaction as observed at biopsy and the level of γ -globulin. In other cases the failure of the liver to destroy γ -globulin, as suggested by Martin (1949), seems inherently possible, for if, as one might reasonably suppose, there is retardation in the liver of both protein anabolism and catabolism, this would account not only for the low level of albumin and α -globulins which the liver synthesizes but also for the high

TABLE II.—Serum Protein Analyses (From Mider *et al.*, 1950)

Diagnosis	Total	Albumin	Globulin			
			α -1	α -2	β	γ
Normal	6.52	4.04	0.38	0.66	0.76	0.66
Cancer	6.02	2.94	0.53	0.90	0.89	0.75
Late cancer	5.73	2.38	0.65	1.05	0.99	0.82

*Of all the boundaries of the electrophoretic spectrum, that between the β and γ is notoriously the least well defined. Extrapolation of the ungeometrical curves which form the closely juxtaposed humps in a phoretogram is capable of large inaccuracies, and so is the arbitrary division of the paper strip with scissors. A second possibility is the presence of a small quantity of denatured protein or of protein adsorbed to the filter paper, which, in the method of Flynn and de Mayo, remains in the fast edge of the γ -band.

level of proteins of extrahepatic origin—that is, γ -globulins. Viewed in this light the customary facile explanation of “a compensatory increase” of globulin contains some sense.

Summary

Electrophoresis of serum proteins in 38 cases of ascites revealed distinct and constant patterns in the major groups with hepatic fibrosis or carcinomatosis peritonei. Other conditions, which form a minority of cases, produce less characteristic changes.

The pathological processes underlying these changes are discussed, and the diagnostic value of serum electrophoresis in this context is assessed.

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MULTIPLE VISCERAL LESIONS DUE TO PHENYLBUTAZONE TOXICITY

BY

J. R. NASSIM, B.M., F.R.C.P.

Physician, St. George's and the Royal National Orthopaedic Hospitals, London

AND

THOMAS PILKINGTON, M.D., M.R.C.P.

Medical First Assistant, St. George's Hospital, London

During the past year many reports have appeared stressing the toxic effects of phenylbutazone. Among the most important of these effects have been agranulocytosis, reactivation of peptic ulcer, jaundice, renal lesions, skin rashes, and oedema (Kuzell *et al.*, 1952; Cudkowicz and Jacobs, 1953; Etes and Jacobson, 1953; Stifel and Burnheimer, 1953).

We have recently seen a patient in whom the drug caused multiple visceral lesions and posed problems in differential diagnosis.

Case Report

A 53-year-old woman attended another hospital in January, 1953, because of severe pain in the right hip due to osteoarthritis. Treatment with analgesics and physiotherapy gave no relief. In April she was admitted to hospital, where she derived some benefit from femoral traction. However, soon after discharge the pain increased and she was given phenylbutazone, 200 mg. thrice daily. A few days later she developed diarrhoea, diagnosed by her own doctor as gastro-enteritis, and treated with kaolin mixture; phenylbutazone was stopped. After the diarrhoea had ceased the patient again took the drug, as it had given

her great symptomatic relief. Within a few days she noticed increasing swelling of her feet and legs, and, soon after this, complained of anorexia, headache, pains in the limbs, substernal pain, and fever. Phenylbutazone was again stopped, but her fever and general condition did not improve. On readmission to hospital on June 11, 1953, she was found to be very ill. Her temperature fluctuated between 99 and 101° F. (37.2 and 38.3° C.), the conjunctivae were suffused, a slight erythematous rash was present on forehead and chest, and there was a maculo-papular rash on both shins: the legs were moderately oedematous and the blood pressure was 100/70. There was slight anaemia (Hb 67%) and a sedimentation rate of 65 mm./hr., but the white blood count was repeatedly normal. The urine contained albumin, a trace of bile pigment, a marked excess of urobilinogen, and granular casts. A tentative diagnosis of leptospirosis was made at this stage, but repeated agglutination tests remained negative. On re-testing the urine ten days later, it was found to contain large quantities of sugar, and a glucose-tolerance test showed a typical diabetic curve; serum amylase levels were repeatedly normal. She was given 12 units of globin insulin daily until the glycosuria had disappeared 10 days later.

An E.C.G. taken on June 23 because of the chest pain showed: “Sinus rhythm 60/min.; horizontal heart; T waves low in AVL, I, and inverted in V₃₋₆, consistent with non-specific myocardial changes.” Radiographs of the chest remained normal. Repeated liver function tests showed a reversed A/G ratio (3.4 g./4.5 g.), rising thymol turbidity, and positive flocculation reactions.

The diagnosis now seemed to be between viral hepatitis and phenylbutazone poisoning. A liver biopsy (see Fig.) was therefore taken, on which Professor Crawford reported: “Sections show a moderate degree of fat accumulation in the liver cells. Individual cells contain large droplets of fat, and the affected cells are irregularly distributed without any special lobular arrangement. There are numerous foci of focal necrosis into which infiltration with mononuclear cells has occurred, and there is also some accumulation of mononuclear cells in the portal tracts. These appearances suggest toxic damage to the liver, and are consistent with the suggestion that the lesion is due to phenylbutazone.” This finding led to the final diagnosis of poisoning with phenylbutazone.

During the second week in hospital the patient's condition improved: the fever subsided and she lost her

Liver biopsy, showing area of focal necrosis with round-cell infiltration. (H. and E. $\times 200$.)