

# ELECTROPHORETIC ANALYSIS OF SEROUS PLEURAL EFFUSIONS

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Since Tiselius (1930, 1937) introduced moving boundary electrophoresis for analysis of protein, and more particularly since paper was used as the suspending medium (Cremer and Tiselius, 1950; Durrum, 1950; Flynn and de Mayo, 1951), many papers have been published describing the changes in the electrophoretic fractions of serum globulin in various diseases. On the other hand little attention has been paid to the electrophoretic analysis of serous effusions.

Luetscher (1941) found a low protein concentration in serous effusions due to nephrosis (0.1 to 0.7 g.%) and portal cirrhosis (0.3 to 0.6 g.%); in congestive heart failure the albumin was proportionately higher and the  $\beta$  globulin lower than in the serum. In lobar pneumonia the protein content was 3.3 to 4.0 g.%, the albumin/globulin (A/G) ratio being higher than in the serum, but in no case was there any evidence of selective secretion of any single protein fraction. Analysis of the protein of tuberculous effusions reflected the variations from normal found in the plasma protein. The analysis of the protein of serous effusions in carcinoma was found to depend on the means by which the fluid was produced; where the pleura was involved the fluid and blood proteins were nearly identical, but if the veins or lymphatics were obstructed the fluid tended to be more dilute resembling that of cardiac failure. The  $\beta$  globulin and fibrinogen were generally found to be less in effusion fluid than in plasma, and it was thought that the association of  $\beta$  globulin with lipid, often in particles of colloidal dimensions, might explain its lowered proportion. The defibrinating action of the pleura probably accounts for the relatively lower amounts of fibrinogen (Denny and Minot, 1916). Serial taps produced little change in the analysis.

Kay (1954) found that electrophoretic analysis of serum was of value in distinguishing the cause of ascites; he studied 38 cases with carcinoma, hepatic cirrhosis, and constrictive pericarditis, but

analysed the fluid in only five. He found, as did Luetscher, that the fluid resembled diluted serum with a somewhat higher A/G ratio.

## METHOD

Whatman No. 1 filter paper in strips 5 cm. wide was used as the suspending medium in a "horizontal" apparatus similar to that first described by Grassmann, Hannig, and Knedel (1951). Serum or pleural fluid was applied with a fine camel-hair brush along a lightly pencilled line across the strip 12 cm. from one end, leaving 1 cm. clear at each side. The buffer solution used was diethyl barbiturate at a pH of 8.6 and ionic strength of 0.1 (Longworth, 1942). A direct current of 2 mA. was passed through each strip for 18 hours. The paper was then removed and dried in a horizontal position at 110° C. for 15 minutes, thus fixing the protein which was then stained by bromphenol blue and the background washed clear with 0.5% acetic acid. The density of the various bands was then read on a semi-automatic recording densitometer similar to that described by Latner, Molyneux, and Rose (1954). The areas below the curves were measured by planimetry and the proportions of the electrophoretic fractions calculated.

## MATERIAL

The groups examined consisted of 64 patients. Twenty-one had malignant effusions, 19 of them having bronchial carcinoma; extensive neoplastic involvement of the pleura was found in five who were examined at necropsy, in seven the pleura was very obviously thickened to needling, and in two the effusion, originally clear, became blood-stained. Fourteen had acute tuberculous effusions of recent onset, and eight had chronic tuberculous effusions (an effusion was classed as chronic when it had been present for more than three months). Four had effusions due to pulmonary infarction; five had effusions following "non-specific" pulmonary inflammation; eight had effusions after treatment of spontaneous pneumothorax by the introduction into the pleura of  $\frac{1}{2}$ % camphor in oil or talc and iodine powder; two had congestive heart failure and two multiple myelomatosis.

## RESULTS

In all groups the average A/G ratio was greater in the pleural fluid than in the serum, but the difference was less in the acute tuberculous group. The globulin pattern of the 14 acute tuberculous fluids was very similar to that of the corresponding serum. All four globulin fractions stained clearly in each case, and in particular the  $\alpha_2$  band was not reduced out of proportion to the others (Fig. 1).

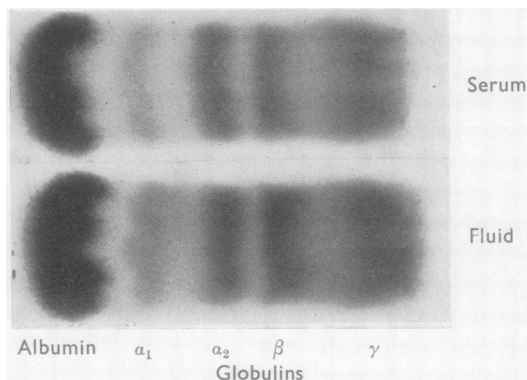


FIG. 1.—Electrophoretic strips of serum and pleural fluid from a patient with an acute tuberculous effusion.

This was not so with the chronic tuberculous or malignant effusions; nor was it so with the effusions following pulmonary infarction or spontaneous pneumothorax. In all these the  $\alpha_2$  globulin band was disproportionately reduced. Typical stained strips from patients with malignant, chronic tuberculous, post-spontaneous pneumothorax, chronic inflammatory, and post-infarction effusions are shown in Figs. 2 and 3. The proportion of the total globulin formed by the  $\alpha_2$  fraction was calculated for each fluid and serum. The results are summarized in Table I.

The number in the pulmonary infarction group is insufficient for statistical analysis. Of the five

TABLE I  
 $\alpha_2$  GLOBULIN CONTENT OF SERUM AND PLEURAL FLUID  
EXPRESSED AS PERCENTAGE OF TOTAL GLOBULIN

Type of Effusion	No. of Patients	Average $\alpha_2$ Globulin	Range	Significance of Difference between Fluid and Serum
Malignant	21	Serum 25.4	21.2-34.4	P=less than 0.001
		Fluid 19.0	11.5-30.3	
Acute tuberculous	14	Serum 24.5	14.1-31.9	P=0.8-0.9
		Fluid 24.1	14.7-31.8	
Chronic tuberculous	8	Serum 25.9	20.3-35.6	P=less than 0.01
		Fluid 18.2	13.0-26.1	
After spontaneous pneumothorax	8	Serum 25.5	21.9-30.5	P=less than 0.01
		Fluid 20.1	16.4-26.1	
After pulmonary infarction	4	Serum 23.1	17.1-29.4	Insufficient in number
		Fluid 17.8	11.9-25.7	

patients in the non-specific inflammatory group, two had effusions associated with bronchiectasis and one had thickened pleura; in these cases the  $\alpha_2$  globulin fraction in the fluid was disproportionately reduced. Two others had serous syn-pneumonic effusions and the globulin fractions in the fluid were very similar to the serum globulin fractions. The reduction in the proportion in the fluid as compared with the serum is statistically significant ( $P < 0.05$ ) in all the other groups except the acute tuberculous group where the difference is not significant ( $P = 0.8-0.9$ ).

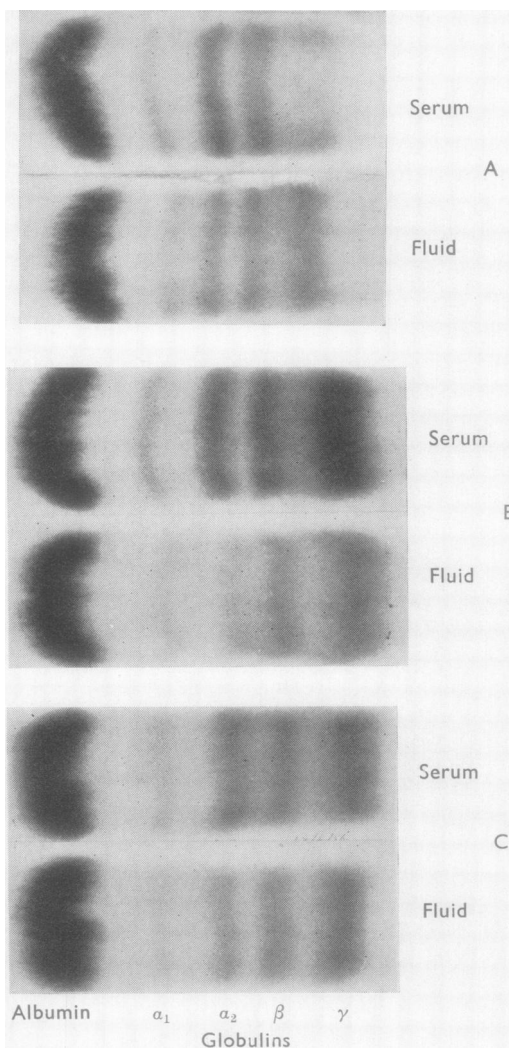


FIG. 2.—Electrophoretic strips of serum and pleural fluid from patients with effusions due to: A, bronchial carcinoma; B, chronic tuberculosis; C, following spontaneous pneumothorax.

There was no statistically significant reduction in any of the other electrophoretic globulin fractions in any of the aetiological groups.

Only two cases of effusion due to congestive heart failure were investigated. This small number is due to the special population from which the cases were drawn. In each case the pleural fluid protein was low and the A/G ratio relatively high. It was found impossible to obtain electrophoretic strips clear enough to scan without concentrating the protein in the pleural fluid. When that was done by dialysing against 20% dextran, to about half volume, clearly stained bands were obtained in all the globulin positions. The globulin fractions appeared in approximately similar proportions to those found in the serum.

There were two patients with multiple myelomatosis. One had an abnormally large amount of protein in a position between the  $\alpha_2$  and  $\beta$  globulin

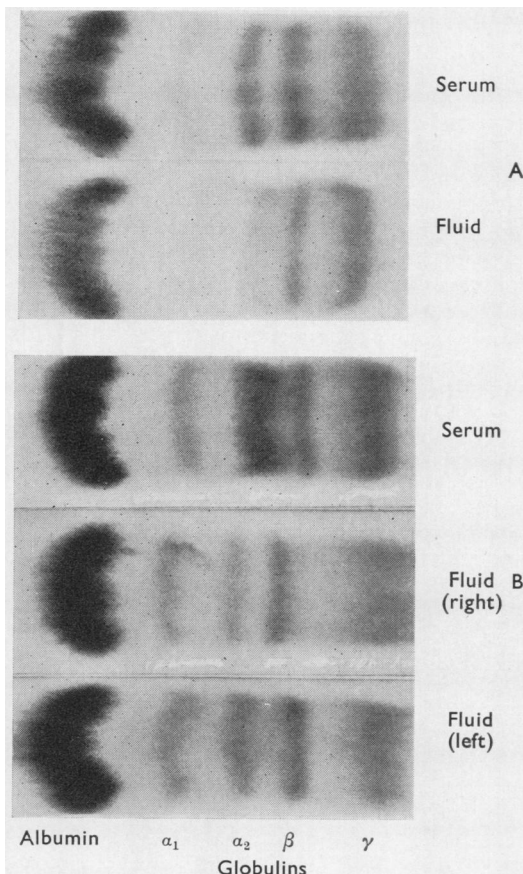


FIG. 3.—Electrophoretic strips of serum and pleural fluid from patients with effusions following: A, chronic non-specific pulmonary infection; B, pulmonary infarction (bilateral).

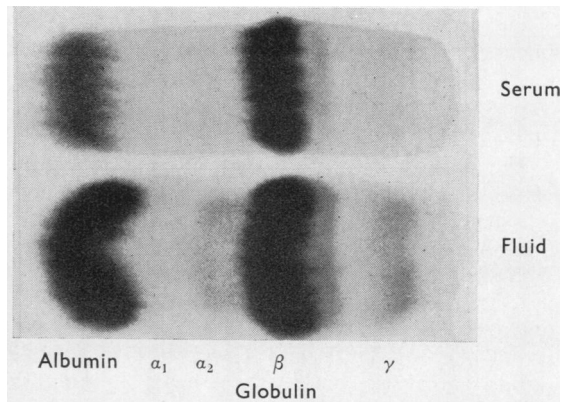


FIG. 4.—Electrophoretic strips of serum and pleural fluid from a patient with multiple myelomatosis.

bands, the other in the  $\gamma$  globulin position. Electrophoresis (Fig. 4) showed that the abnormal protein appeared in the pleural fluid in much the same proportion as in the serum. Electrophoresis of the urine in one of the cases showed a similar band, but the protein was not in sufficient amount to make scanning possible without dialysis, which was not attempted.

Five patients (all with bronchial carcinoma) had fluid analysed again after an interval of two months or more and there was no significant change in any of the globulin fractions. In two, the total amount of protein in the pleural fluid had fallen, but the proportions of the various fractions remained largely unaltered.

#### DISCUSSION

In these electrophoretic analyses, the proteins of acute tuberculous effusions followed quite closely the serum proteins. This was so in the only two other "acute" serous effusions examined. This finding agrees with that of Luetscher (1941). In malignant effusions, chronic tuberculous effusions, and those following chemical irritation of the pleura for the treatment of spontaneous pneumothorax, the  $\alpha_2$  fraction was regularly reduced disproportionately as compared with the serum, the percentage reduction being statistically significant. The other globulin fractions were demonstrated in much the same proportion in the pleural fluid and in the serum.

In the pleural fluids the albumin/globulin ratio was persistently increased as compared with the serum ratio, but the increase was less in the acute tuberculous effusions. This suggests that the protein content of an effusion depends quite substantially on the state of the associated capillaries.

The reduction in the  $\alpha_2$  globulin fraction was an unexpected finding; Luetscher (1941) reported that the  $\beta$  globulin fraction was reduced more than the others because it contained protein-bound lipids. His results are not confirmed by this study.

The reason for the disproportionate reduction of the  $\alpha_2$  fraction is not known. The molecular weight of the various proteins making up the  $\alpha_2$  fraction is not greater than that of the other globulin fractions, and thus reduced filtration is unlikely to be the cause; but little is known about the size of the particles in the blood stream, and recently Exton-Smith and Crockett (1957) have stated that absent or greatly reduced  $\alpha_2$  globulin (0.1 to 0.9 g. per 100 ml.) characteristic of low-protein oedema, is found in all types of oedema in which the capillary permeability is believed to be normal.

Only two effusions due to congestive heart failure were examined. In neither was the  $\alpha_2$  globulin disproportionately reduced. Although the protein content of each fluid was only 2 g. per 100 ml. the pleura was not obviously thickened and the capillary permeability may have been greater than normal.

The reduction of the  $\alpha_2$  fraction might be due to selective deposition of all or part of it on the walls of the space, and this fits in with the fact that in most instances where the  $\alpha_2$  globulin was reduced the pleura was substantially thickened. Thickening was not a notable feature after spontaneous pneumothorax, but all these patients had either 0.5% camphor in oil or talc and iodine powder sprayed over the pleural membranes, with subsequent formation of fibrinous adhesions.

The reduction in  $\alpha_2$  globulin in a pleural fluid when compared with the corresponding serum is of little value in differential diagnosis, except that it can be said that if the serum/fluid  $\alpha_2$  globulin ratio is greater than 1.3 the effusion is unlikely to be an acute tuberculous effusion.

No attempt was made to estimate the absolute amount of albumin or the various globulin fractions by electrophoresis. The changes occurring in the serum proteins in pulmonary tuberculosis have been fully reported by Seibert, Seibert, Atno, and Campbell (1947), Baldwin and Iland (1953), and Gilliland, Johnston, Stradling, and Abdel-Wahab (1956). Changes occurring in other diseases have been reported by Seibert and others (1947), Flynn (1954), Hardwicke (1954), and many others. Further, Martin and Franglen (1954) have shown that paper-strip electrophoresis cannot properly be used for quantitative analysis.

Gilliland and others (1956) found that the albumin/ $\alpha_2$  globulin ratio could be correlated with the activity of pulmonary tuberculosis, varying from 3.45 for patients with extensive active tuberculosis to 5.69 for those with arrested disease. The albumin/ $\alpha_2$  globulin ratio of the serum was calculated for the electrophoretic analyses reported here. The mean values were 2.4 for patients with malignant disease, 2.7 for those with acute tuberculous pleural effusions, 3.3 for patients with chronic tuberculous effusions, and 3.0 in the patients with spontaneous pneumothoraces. The mean differences were not significant. The albumin/ $\alpha_2$  globulin ratios in the present investigation were smaller than those of Gilliland and others (1956), probably because, in order to get clear staining of the globulin fractions, the amount of protein applied on the paper was greater than the 0.01 ml. which they applied; and the photo-electric scanner tends to give abnormally low readings if the dye in a particular band is too heavy.

#### SUMMARY

The proteins of blood serum and of pleural fluid were analysed by electrophoresis in 64 cases.

The albumin/globulin ratio was regularly greater in the fluid than in the serum.

In malignant and chronic tuberculous effusions, and in those following pulmonary infarction and spontaneous pneumothorax, the  $\alpha_2$  globulin fraction was disproportionately reduced in the fluid.

The pattern of the acute tuberculous effusions closely followed the serum pattern.

Electrophoretic analysis of pleural fluid is of little value in diagnosis of the cause of the effusion.

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