A THESIS

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entitled

Serum Protein Changes in Caplan's Syndrome.

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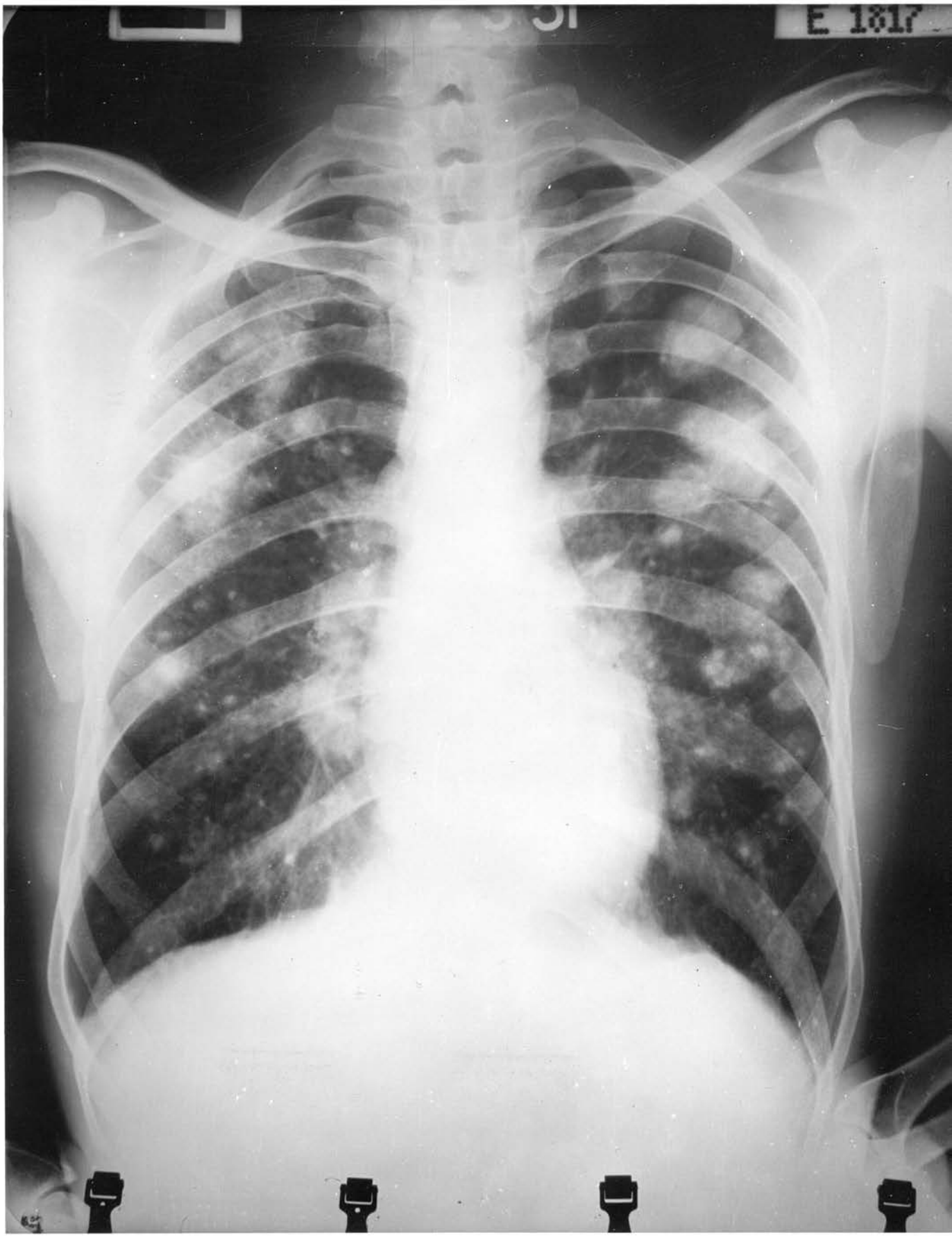
Postero-anterior chest radiograph of a case of Caplan's syndrome. (Grade 3).



INTRODUCTION

The association of rheumatoid arthritis in Welsh coal miners with characteristic radiological opacities in the lungs was first described by Caplan (1953). He claimed that the opacities characteristic of this condition (Fig. 1) differed from Progressive Massive Fibrosis (P.M.F.) in several respects, namely:- (1) They developed more rapidly; (2) they often developed on a background of less severe simple pneumoconiosis; (3) they were small, round and well defined even in an early stage when P.M.F. would be diffuse and irregular in outline; (4) they were usually numerous compared with P.M.F. lesions, and, (5) though chacacteristically peripheral in distribution they affected all zones of both lungs with equal frequency whereas P.M.F. favours the right lung and the upper zones.

The aetiology of these pulmonary lesions remains obscure.

The syndrome was investigated by Miall, Caplan, Cochrane, Kilpatrick and Oldham (1953). More than half of the 20 subjects whose x-rays were picked out by Caplan as characteristic from a group of 896 previously classified as P.M.F. or tuberculosis, were found to have rheumatoid arthritis compared with 3% of the control group. It was noticed that the development of the lung lesions might either precede or follow the development of arthritis by several years.

A further investigation was undertaken (Miall 1955) to determine whether P.M.F. was an aetiological factor in the development of rheumatoid arthritis, since even 3% is signi-

ficantly higher than the incidence in the general population. In a geographically defined community of 9,430 males over the age of 15, where P.M.F. and Caplan's Syndrome were prevalent, no increased prevalence of rheumatoid arthritis was demonstrated amongst miners and ex-miners. A significantly high incidence of P.M.F. and Tuberculosis was found, however, among the miners with arthritis and a significantly high incidence of Tuberculosis amongst arthritic non-miners. This investigation also confirmed that genetic factors are of importance in the aetiology of both rheumatoid arthritis and Caplan's syndrome. A hypothesis was proposed that the development of rheumatoid arthritis and of "Caplan" lung lesions had a common origin in an inherited abnormality of tissue reaction which might also predispose to the development of tuberculosis.

A number of whole lungs removed post mortem and two lung biopsy specimens from patients with pneumoconiosis and rheumatoid arthritis have now been examined, and Gough, Rivers and Seal (1955) published the findings in 16 cases. Six of these (38%) showed some evidence of past or present tuberculosis which is a similar incidence to that found at necropsy in cases of P.M.F. (Rivers et al 1957). In four of the cases with no evidence of active tuberculosis areas of active inflammation were found within the nodules which did not resemble tuberculosis and which are not found in P.M.F. lesions. This inflammation was tentatively identified as the "rheumatoid component". A further point of interest emerging from the pathological study was that some cases recognisable as examples of Caplan's syndrome from a whole lung section (Fig. 2) might not have been so recognised from a postero-anterior chest radiograph because coalescence or

FIGURE 2

Section of a whole lung from a case of Caplan's syndrome. (Grade IV).

superimposition of discrete nodules produces an appearance indistinguishable from P.M.F.

One of the rheumatoid lung lesions removed at thoracotomy was studied chemically by Consden and Glynn (1955). The nodule showed no histological evidence of tuberculosis and was negative on culture and guinea-pig inoculation. Two dimensional chromato-ionography of tissue hydrolysates demonstrated, however, the presence of α-ε-diaminopimelic acid (D.A.P.) in about one third the concentration found in caseous tuberculous tissue. This amino-acid is not found in normal mammalian tissue, neither were Consden and Glynn able to detect it in P.M.F. lesions.

Consden and Howard (1957) examined a further Caplan nodule by a new and improved technique and again found D.A.P. They again failed to demonstrate this amino-acid in P.M.F. in spite of the increased sensitivity of the method. Nethercott and Strawbridge (1956 i) claim, however, to have detected D.A.P. by the method of Consden and Glynn in 14 out of 15 cases of P.M.F. These workers then investigated other lesions including those of Sarcoidosis (Nethercott and Strawbridge, 1956 ii) and Caplan's syndrome (unpublished work) by the same technique subsequently extracting the humin residue from the hydrolysis to obtain, in every case, an acid-fast, lipoid substance which they claim to have identified as mycolic acid. This identification has been disputed by Consden (1957) and by Berg (1957) but is accepted by Asselineau (personal communication). Should the claims of Nethercott and Strawbridge be substantiated the tuberculous theory of the aetiology of P.M.F. and Caplan lesions would be greatly strengthened since mycolic acid is much more specific to the tubercle bacillus

than is D.A.P. which occurs in numerous other common bacteria; according to Asselineau (1951 and personal communication) mycolic acid can be extracted from all strains of Mycobacterium so far tested including atypical strains of M.Tuberculosis. Only saprophytic strains of M. Leprae had been studied and Actinomycetes had not as yet been investigated. Asselineau does not accept Berg's identification of mycolic acid in mammalian semen and knows of no other case in which a mammalian origin has been claimed.

The evidence to date points to a tuberculous aetiology for the lung lesions described by Caplan but it can scarcely be considered conclusive. Seibert, Seibert, Atno and Campbell (1947) showed that in pulmonary tuberculosis an increase in serum γ-globulin occurs in the early stages and that in the more advanced cases α2-globulin increases. Gilliland, Johnston, Stradling and Abdel-Wahab (1956) used the protein changes as a measure of tuberculous activity and showed that the ratio of albumin to α2-globulin was a more reliable index than the Erythrocyte Sedimentation Rate (E.S.R.). Christiaens, Balgaires, Claeys and Lenoir (1954) found similar changes in coal-workers' pneumoconiosis only when tuberculosis was also present but claimed that a rise in γ-globulin occurred in the absence of tuberculosis although the increase was small. Poyard and Nizet (1955) denied that γ-globulin was raised but affirmed that in advanced cases α2-globulin was increased even in the absence of demonstrable tuberculosis. In silicosis and particularly silico-tuberculosis Beckman, Antweiler and Hilgers (1953) found a reduction in albumin and an increase in γ-globulin the most striking feature but Rosenkranz (1957) found that in silico-tuberculosis the rise in α2-globulin and fall in

albumin were the most constant changes and showed the greatest difference from pure silicosis. Cases under treatment showed a return of the protein pattern towards normal as the clinical condition improved as also did the series of tuberculous patients described by Gilliland et al. (1956). Barhad, Vlad and Dron (1956) describe changes in albumin, α2- and γ-globulins in rabbits with experimental silicosis. Shaw (1956) claims that especially large increases in α2-globulin occur in the presence of tuberculous pleural effusion and Prignot (1956) states that the α2-globulin reaches its highest level in miners at the time cavitation of P.M.F. occurs.

Thus there appears to be general agreement that tuberculous conditions give rise to low albumin and high α2-globulin levels, opinions being divided on the question of γ-globulin. Unfortunately, these changes are in no way specific, being found in numerous other chronic inflammatory conditions and in carcinomatosis, renal and hepatic diseases. Rheumatoid arthritis itself causes a reduction in albumin and increases in α2- and γ-globulin (Ropes, Perlman, Kaufman and Bauer, 1954) (Kuhns and Crittenden, 1955) though Salt (1956) claimed to find normal protein patterns in nearly half his patients and γ-globulin more often increased than α2. His group included cases of inactive rheumatoid arthritis, osteo-arthritis and fibrositis, but it is not clear whether these were the subjects in whom normal protein-patterns were found.

It was realised, therefore, that the rheumatoid arthritis would in many cases cause serum abnormalities but it seemed probable that if "Caplan" lung lesions were tuberculous they would have an effect upon the proteins additional to that due to rheumatoid arthritis. As the condition is an

active and rapidly progressive one compared with P.M.F., giving rise to cavitation earlier and more often (Caplan [1959]), and not infrequently associated with pleurisy and pleural effusion, it seemed reasonable to expect its effect upon the serum proteins to be large enough to be detected even in the presence of rheumatoid arthritis.

It was therefore decided to undertake an investigation of the serum proteins of a group of patients selected to cover as far as possible the whole spectrum from mild rheumatoid arthritis with little, if any, pneumoconiosis to advanced cases of Caplan's syndrome, with a view to discovering whether the lung pathology characteristic of the latter gives rise to serum protein changes resembling those occurring in tuberculosis.

SELECTION OF SUBJECTS.

A. MINERS.

The patients studied consisted of 50 Welsh coal-miners, all of whom had been exposed to a significant dust hazard although two failed to show any radiological evidence of pneumoconiosis. All were patients admitted to Llandough Hospital for treatment or attending our outpatient clinic. All such patients who had clinically diagnosable rheumatoid arthritis or typical "rheumatoid" lung lesions with or without arthritis were included in the series, except those whose chest radiographs showed lesions resembling orthodox P.M.F.; these were excluded unless previous X-rays or tomography revealed a definite nodular picture such as Caplan described. Those whose radiographs showed the earliest detectable stage of P.M.F. ["A" shadows on the I.L.O classification (Drinker, 1954)]were included on the ground that these lesions might develop into "Caplan" nodules and would then be of particular interest in the follow-up study which was planned. Of the 50 patients 5 were later excluded from the series, 2 because of other diseases likely to affect the protein pattern, 2 because of doubt as to the nature of lung lesions on the basis of which they had been included, and one because of doubt as to the diagnosis of rheumatoid arthritis.

B. NORMALS.

A group of 12 normal subjects between 40 and 49 was also studied to provide information as to the values to be expected for the various protein fractions in healthy individuals. The 40 to 49 age-group was chosen because this is the largest 10-year age-group in the series of 45 patients.

METHOD OF INVESTIGATION.

The patients were investigated in the following 7 ways:-

1. Industrial history to establish exposure to a real dust hazard.
2. Medical history including family history.
3. Clinical examination.
4. E.S.R. (Westergren at 1 hour).
5. Differential Agglutination Test (D.A.T.) by the method of Ball (1950).
6. Serum Electrophoresis by the method described below
7. P-A. Chest x-ray supplemented by laterals and tomograms when thought necessary.

The first 4 investigations were done on the same day in every case and blood for electrophoresis and D.A.T. was obtained simultaneously; the serum was separated and stored, frozen solid, until required. Chest x-rays were taken on the same day of all out-patients and on admission in the case of in-patients in whom the remaining investigations were usually done several days later.

Haematological investigations were performed on all in-patients but on out-patients only when there was clinical evidence of anaemia. Joints were x-rayed in the majority of cases, but the x-rays were used in diagnosis only when other investigations left this in doubt.

ELECTROPHORETIC METHOD.

At the time this study was conceived the literature on electrophoresis of serum proteins, though already copious, did not include a complete description of any method which fulfilled the author's requirements. The method described by Gilliland et al. (1956) had the advantages of speed, simplicity and cheapness, but its validity had not been fully established.

An investigation was therefore undertaken to assess Lissamine Green as a protein stain in terms of nine criteria which it was felt a dye must fulfil if it is to be suitable for routine use in paper electrophoresis. The dye must:-

(1) give consistent results from one batch to another,

(2) stain protein but not paper,

(3) stain all proteins equally,

(4) retain a linear Dye/protein relationship up to the maximum concentration of protein likely to be encountered,

(5) be readily eluted in a non-volatile solvent,

(6) be stable under conditions of use,

(7) give the same results by elution and by direct densitometry,

(8) be insoluble in clarifying agents used in direct densitometry,

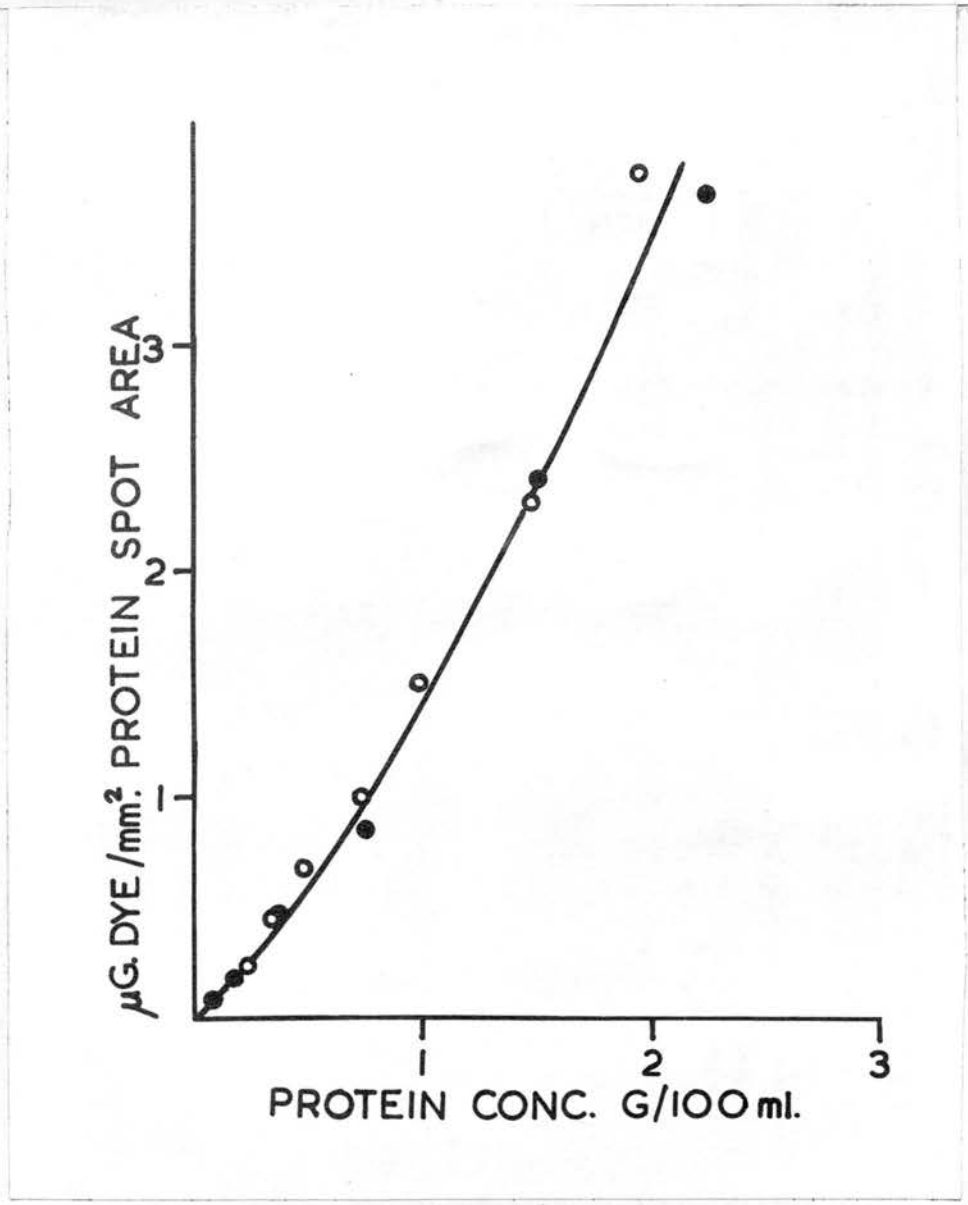
(9) be quick, cheap and simple to use

The results of this investigation have been published separately (Gorringe, 1957) and can be briefly summarised as follows:-

(1) Notwithstanding slight chromatographic dissimilarities (less marked than those reported by

FIGURE 5.

Relationship of Dye Density to Protein Concentration.



Martin [1956]) the seven batches of lissamine green tested behaved similarly in every respect as also did one batch tested after repeated use.

(2) Lissamine green stains protein but leaves the paper perfectly white.

(3) At concentrations in which they occur in paper electrophoresis the dye-uptake of albumin, γ-globulin and the mixed proteins of whole serum is similar.

(4) The dye-uptake of albumin is proportional to concentration to the maximum investigated; this corresponded to a density on the paper of $14{\cdot}23 \times 10^{-4}$ mg./mm^2. which exceeds the maximum likely to be encountered. The γ-globulin/dye characteristic begins to flatten out at high concentrations but also remains linear up to the maximum density likely to occur in practice. Figure 3 relates the two vital parameters, concentration of protein solution applied to paper, and corresponding dye-density after staining, showing a near-perfect relationship.

(5) Elution of lissamine green is quick and simple and, though not quite complete, is the same for all proteins within the working range of concentrations.

(6) Lissamine green is stable in aqueous solution at pH 6·0 and below. There is no need to use a higher pH.

(7) Scanning with a densitometer does not produce the same results as elution, tending always to under-estimate albumin. When the Albumin-Globulin (A/G) ratio is known, however, the values obtained by densitometry can be corrected to give results corresponding closely to those obtainable by elution. The elution technique can be used if

desired to determine the A/G ratio.

(8) Lissamine green is insoluble in the clearing fluid used for densitometry and in ether used to remove the clearing fluid.

(9) The lissamine green staining technique described is quick, cheap and simple.

Many of the above observations have been confirmed by Brackenbridge (1960). Failure to confirm others may be due to the fact that Brackenbridge used a solution of lissamine green incorporating salicylsulphonic acid to denature the protein, whereas heat denaturation (1 hour at 100° C) was used throughout the above investigation.

Clearly lissamine green comes close to fulfilling all of nine criteria except number 7, and its failure in this respect is certainly a fault of the supporting medium rather than of the dye. This one defect in the technique could no doubt be corrected by the use of a homogeneous, transparent supporting medium such as agar gel on cellophane (Giri, 1956) or cellulose acetate (Kohn, 1957), but for the purposes of the present investigation it was decided to use the elution technique throughout and dispense with direct scanning, except as an aid to dividing the strip reproducibly into segments corresponding with protein bands. (See p.13 below).

APPLICATION OF METHOD

Electrophoresis was carried out using 5 x 36 cm. strips of Whatman No. 1 paper in a closed horizontal tank. The electrode compartments contained barbitone/acetate buffer, pH 8·6, and ionic strength 0·1. To ensure uniform wetting of the paper, only the ends were wetted, the remainder being

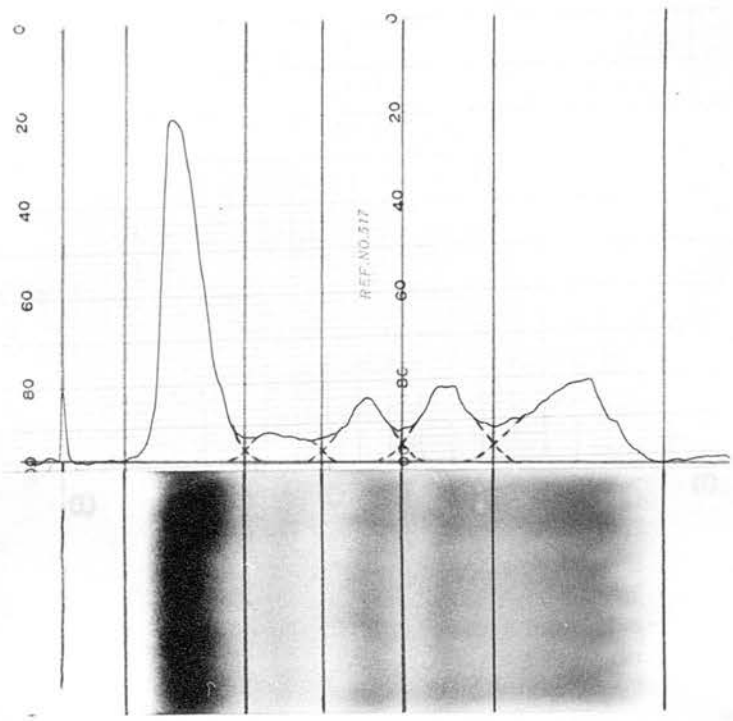
allowed to wet iself slowly by capillary action. 0·01 ml. of serum was applied along a line across the paper about one-third of the way from the cathode end by means of a glass applicator slightly shorter than the width of the paper, and a current of 1 mA per paper passed for 18 hours at a room temperature of 70° F.$\pm 5^{\circ}$. After the run the ends of the papers were cut off with a knife to prevent distortion by the "hanging-drop effect" (Williams, Pickels and Durrum [1955]) and the horizontal part removed as quickly as possible, suspended horizontally over a tray by means of clips at either end and placed in an oven at 100° C for one hour

Staining was carried out in a similar manner to that described by Gilliland et al., (1956). The papers were laid flat in a stainless steel tray, and 0·3% lissamine green S.F. 150 in 15% acetic acid in tap water was poured over them. The tray was agitated sufficiently to remove air bubbles from beneath the papers, and staining allowed to proceed for 10 min. The stain was decanted for re-use and the papers were then covered with 2% acetic acid in tap water and rocked for 2 min. The washing fluid was poured off, and two further 2-min. washes were given with similar fluid. At the end of this procedure the background was perfectly white and the papers were hung up to dry at room temperature.

The dry strips were rendered translucent with the clearing fluid described by Rees and Laurence (1955) and scanned in a recording densitometer (Laurence, 1954) using a compound filter consisting of one thickness of Ilford No.205 gelatin and one thickness of Chance heat-resisting glass.

FIGURE 4.

Densitometer trace with the corresponding stained strip showing the method used for dividing the strip reproducibly into bands corresponding to protein fractions.



The trace produced was used as a template for dividing the stained strip reproducibly into 5 segments corresponding to the 5 protein fractions. This was done by completing the curves, dropping perpendiculars through the points of intersection onto the baseline and then transferring these lines on to the stained strip, which was positioned over the trace by means of a pencil line drawn on the strip beyond the albumin band before scanning and so represented on the trace by a sharp peak (Fig. 4). Each stained strip was then divided longitudinally into equal halves by another pencil line and cut up into 10 segments. The 5 segments in each half paper corresponding to the 5 protein fractions were eluted separately and estimated in a spectrophotometer at 640 mμ. The two sets of optical densities obtained were compared, and if compatible, added together. The value for each fraction was then expressed as a percentage of the total. The object of eluting each paper in two halves was twofold; in the first place it was necessary to keep the highest optical density within the accurate range of the spectrophotometer. This could have been achieved by using twice the quantity of eluting fluid or thinner cuvettes, but it was felt that duplicate estimations would constitute a useful check against gross error.

Total protein estimations were done by the specific gravity method (Phillips, Van Slyke, Dole, Emerson, Hamilton and Archibald [1944 and 1945]), but there seemed no object in expressing protein fractions in absolute concentrations since this practice tends to obscure differences by making all the values numerically small. No relationship was found between total protein values and severity of disease so that percentage values have the same validity as absolute ones.

DIAGNOSIS AND GRADING

A. Rheumatoid Arthritis.

In order to include mild and inactive cases of rheumatoid arthritis in the study the strict diagnostic criteria of Miall (1955) and of Kellgren and Lawrence (1956) had to be relaxed to some extent but all except two of the more severe cases (Grade 2 and above; see below) had 2 or more of their 3 criteria - history of characteristic polyarthritis plus characteristic radiological joint changes and/or a positive D.A.T. It was felt that the clinical diagnosis was sufficiently certain in the case of the two exceptions to justify their inclusion.

Clinical diagnosis was based on first a history of past or present polyarthritis with morning stiffness, pain in and/or swelling of joints affected, and secondly on clinical examination of all joints, especially those of the hands and feet for tenderness, limitation of movement, crepitus, swelling, redness, heat, effusions, muscle wasting and deformity; examination of the skin for palmar erythema, hyperhydrosis, pigmentation, rheumatoid nodules, psoriasis or other skin disease and examination for enlargement of liver, spleen and lymph-nodes.

All cases in whom rheumatoid arthritis was diagnosed were first graded as Active or Inactive (the latter are subsequently designated "R" - for "Remission"). Remission was inferred when a patient had not more than one sign or symptom in each of the following groups:-

Group 1. (Joints)
- Morning stiffness of one or more joints.
- Pain and/or tenderness in one or more joints
- Swelling of one or more joints.

Functional disability not attributable to residual deformity.

Group 2. (Systemic) Rheumatoid Nodules.

Abnormal fatigue and lassitude

Hyperhydrosis

Palmar erythema

Limitation of movement, crepitus, muscle wasting and deformity were regarded as compatible with rheumatoid arthritis in remission. Hot, red joints and intra-articular effusions were regarded as always indicating activity.

This exclusively clinical assessment of activity was necessitated by ignorance as to the effect exerted upon the E.S.R., white count and possibly other criteria often employed such as the haemoglobin level (Duthie, Brown, Knox and Thompson [1957]), by the lung pathology. The identical mean E.S.R. level found in those without arthritis and those with arthritis graded as inactive (Fig.7) suggests that the assessment was valid.

Cases judged to be active were further classified according to the severity of the disease as follows:-

Grade 1. "Doubtful"

Complaints of pain, swelling or stiffness of joints. No disability and minimal clinical signs.

Grade 2. "Slight"

Complaints of pain, swelling or stiffness of joints with slight disability and mild but definite clinical signs.

Grade 3. "Moderate".

Complaints of pain, swelling or stiffness of

joints with moderate disability and obvious clinical signs.

Grade 4. "Severe".

Severe pain, swelling and stiffness of numerous joints with severe disability and gross clinical signs.

Patients with no past or present history of arthritis were designated "Grade 0".

This grading is similar to that used by Kellgren et al (1956), but there is, in fact, nothing "doubtful" about the diagnosis of the 5 patients in Grade 1, since they consisted of two cases of recent onset (3 weeks and 2 days) both of whom subsequently developed more severe disease and one of whom (the latter) developed rheumatoid nodules simultaneously with his minimal joint symptoms, two others with positive D.A.T.s and one whose disease had formerly been more severe but who could not be classified as a case of remission.

The grades represent points on a continuous spectrum and differences of opinion might well exist between observers as to the grading of any one case. All cases were, however, examined and graded by a single observer. Since each grading represents an opinion at a particular point in time it is not possible to estimate reproducibility but subsequent grading of 20 of the patients who were followed up for periods up to 2 years shows satisfactorily consistent results except when remission has occurred. The distribution of cases by grades in the whole series is shown in Table I.

TABLE I

Distribution of Cases by Grades of Arthritis.

GRADE	0	R	1	2	3	4
No. of Cases.	6	6	5	14	10	4

B. Lung Lesions.

The diagnosis and grading of lung lesions is radiological and presented certain difficulties. In the I.L.O. classification of complicated pneumoconiosis any case in which massive lesions occupy more than three anterior rib spaces on either side comes automatically into Grade "C" and this is true of most cases of Caplan's syndrome because of the usually large number of nodules (Fig. 1). This system was therefore considered unsuitable for classifying Caplan-type chest-radiographs for the purpose of this investigation. A second possibility is the Ministry of Health (1947) zone system for radiological classification of pulmonary tuberculosis. This makes numbers rather small by dividing the cases into 7 groups. Thirdly, a classification simply by number of nodular lesions might be attempted but is difficult to use because coalescence and superimposition make counting unreliable in many cases. A compromise scheme was used and the films were divided into the following five groups:-

0. No massive lesions - Simple pneumoconiosis or normal x-ray.

1. Earliest detectable massive lesions - non-specific "A" shadows.

2. 3 or fewer specific nodules on either side.

3. More than 3 specific nodules on one or both sides.

4. As 3 but with confluence of nodular lesions.

All x-rays were read on the same day by the same observer on two separate occasions several months apart. This scheme of grading yielded 85% reproducibility. The 7 films graded differently on the two occasions were read a third time and an agreed grade arrived at.

Of the 45 subjects 7 had rheumatoid arthritis without characteristic lung lesions (grade 2 or above), 6 had characteristic lung lesions without past or present arthritis and 32 had Caplan's syndrome.

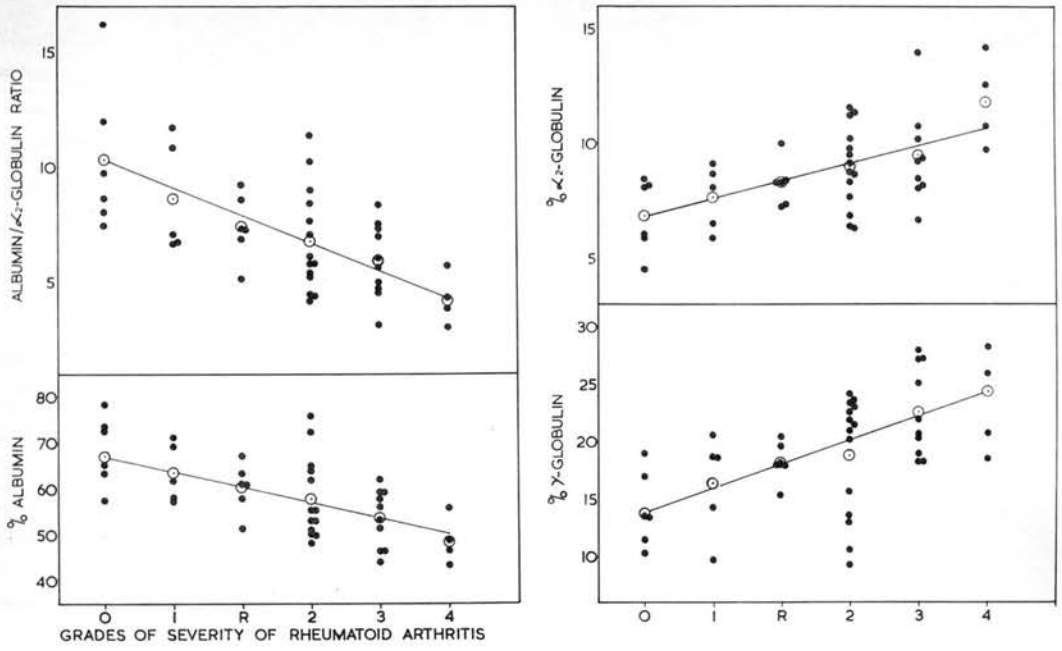


Fig. 5. Scatter diagram showing relationship between severity of rheumatoid arthritis and various protein fractions.

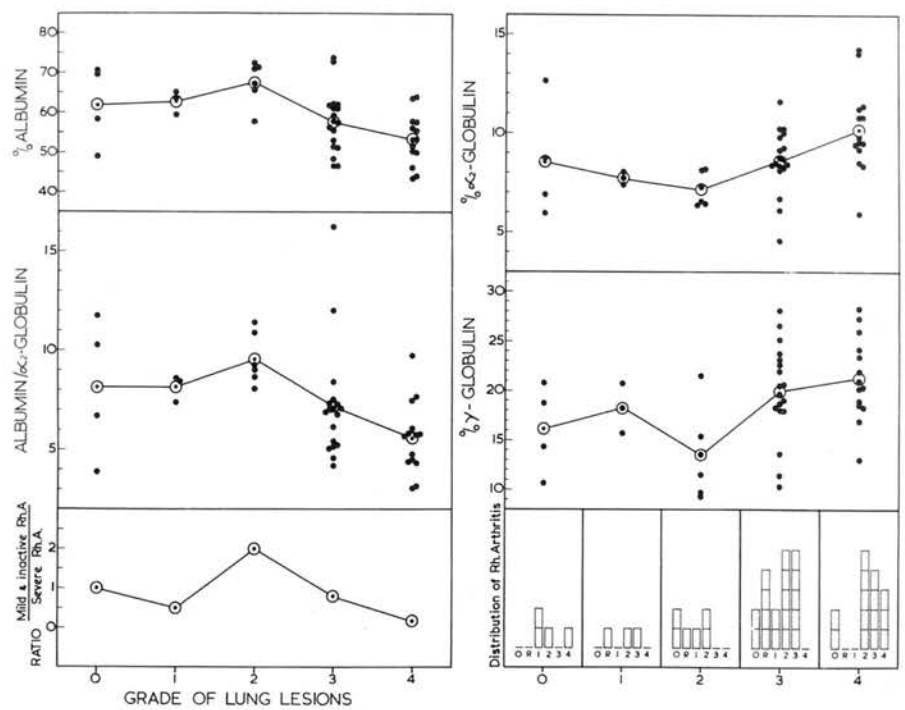


Fig. 6 Scatter diagram relating the extent of lung lesions to protein values. The rheumatoid ratio plotted at bottom left is derived from the histogram by dividing the number in rheumatoid grades 0, R and 1 by the number in grades 2, 3, and 4 for each lung lesion grade.

RESULTS.

The results of the investigation are summarised in Tables II and III which include all the data from which the figures were drawn and conclusions reached.

Fig. 5 shows the relationship between severity of rheumatoid arthritis and the levels of albumin, α2 - and y-globulins and the A/α2 ratio. The remission group values have been inserted between Grades 1 and 2 because this is where their mean seems, in every case, to fit. Considering the small numbers and large scatter, the mean values show surprisingly consistent trends.

When the extent of the lung lesions was similarly plotted against the various protein fractions no such obvious relationship appeared (Fig. 6). In fact, the group with Grade 2 lung lesions show less abnormal protein patterns than those with none. When the rheumatoid structure of each group was examined (see histogram at bottom right of Fig. 6) the fact emerged that, irrespective of the extent of the lung disease, the groups containing the most severe rheumatoid arthritis showed the greatest abnormality of the serum proteins and vice versa. This relationship is even more clearly demonstrated by the curve at bottom left of Fig. 6 which is derived by plotting for each grade the ratio of mild and inactive to severe rheumatoid cases in that group. The shape of the curve so closely resembles the others of Fig. 6 as to raise a doubt whether the lung pathology contributes anything to the serum protein abnormalities.

Before Figs. 5 and 6 can be properly evaluated it is necessary to know whether an association exists between

TABLE II

Summary of Investigation of 50 Patients Excluding Serum Protein Analyses.

Case No.	Lung Lesion Grade	Rh. Grade	E.S.R. Westergren at 1 hour	D.A.T. 1 / Titre at 18 hr.	Nodules	Psoriasis	Family History Rh.	Family History Tb.	Remarks.
1	4	2	32	256	-				L. Basal Pleurisy
2	1	3	47	128	-				Felty's Syndrome. Rubber Allergy.
3	3	3	54	64	-				Allergy to Aspirin, Phenylbutazone and Gold
4	4	4	37	32	-		+	+	
5	3	R	20	16 P.P.	+		+		Encysted L. Basal Effusion for 3 years.
6	2	R	10	< 4	-				
7	4 C	4	38	32	-	+			Died 1 month later.
8	3	R	16	256	+				R. Basal Pleurisy with effusion 2-9/12 before. Lung biopsy 1 yr.before
9	3	2	48	512	- P.P.				
10	4	2	28	8	-			+	
11	4 C	3	25	256	+				Error in E.S.R.suspected. Following week 52 mm.
12	4 C	2	65	64	+				Herpes Zoster at time of examination
13	4 C	3	39	64	+				
14	4 C	3	106	256	+				Familial Ichthyosis. Died 2 months later.
15	4 C	4	55	64	+	+			A.F.B.in Gastric Lavage on one occasion. Virulence unknown.
16	1	R	10	16 P.P.	- P.P.				
17	2	0	3	< 4	-				
18	3	2	40	32	-				
19	Excluded. Other disease - ? Myelomatosis.								
20	4	2	21	< 4	-				Sputum Positive 2 days later. Died 1 year later.
21	3	R	16	64	-				
22	3	2	30	128	-	+			Pleurisy 4 years before. Rh.A in remission from 1930 to 1953.
23	2	0	19	256	-				Cervical Spondylosis causing sensory loss of ulnar distribution.
24	4 Cal	3	42	256	+			+	
25	2	1	3	32	-				Allergy to Penicillin,Streptomycin, P.A.S. and ZnO strapping.
26	3	1	41	8	-				

Case No.	Lung Lesion Grade	Rh. Grade	E.S.R. Westergren at 1 hour	D.A.T. 1 Titre at 18 hr.	Nodules	Psoriasis	Family History Rh.	Family History Tb.	Remarks.
27	2 C	2	39	32	-				
28	4 C	0	24	< 4	? +				Pleurisy, 1946.
29	3 C	2	64	128	+		+		
30	3	3	47	128	+				
31	Excluded. Other disease - Bronchial Carcinoma								
32	0	2	19	< 4	-	+			
33	3 C	2	69	64	- P.P.				Calcified Hilar Lymph Nodes.
34	Excluded. Diagnosis in doubt								
35	1	2	30	64	-			+	
36	0	1	33	< 4	+				Hypertensive. Died 4 months later.
37	0	1	8	< 4	-	+			
38	0	4	57	< 4	? +				
39	4	2	35	256	-		+		Sputum Positive 6 years previously. Urticaria
40	3	3	21	512	+		+	+	Pleurisy at age 21
41	3 Cal	0	9	128	-				
42	3	0	5	32	-	+			D.A.T. formerly as high as 1024.
43	Excluded. Diagnosis in doubt								
44									
45	2	2	23	64	+				
46	3	1	44	64	-				
47	4	0	36	128	-		+		
48	3	R	24	32	+		+		
49	3 Cal	3	104	512	+				
50	3 C	3	68	256	+		? +		1 Son had Rh. Fever and died of Rh. heart disease.

C = Cavitation.
Cal = Calcification.
P.P.= Previously Present or Previously Positive.

TABLE III

Serum Protein Analysis of Forty-Five Patients

Case No.	Total Protein G/100 ml.	Protein Fractions					A/G Ratio	A/α2 Ratio
		A	α1	α2	β	γ		
1	6·66	53·17	6·25	9·17	11·20	20·21	1·135	5·80
2	7·02	59·45	4·50	8·06	7·22	20·75	1·466	7·36
3	6·84	46·55	7·17	10·22	9·60	26·46	1·148	4·55
4	6·84	56·05	4·00	9·77	11·60	18·57	1·275	5·74
5	7·02	61·10	3·46	8·33	9·12	18·00	1·570	7·34
6	7·02	67·23	2·81	7·27	7·33	15·36	2·050	9·25
7	7·20	43·45	4·74	14·23	11·61	25·95	0·770	3·05
8	7·20	51·50	5·70	10·01	12·34	20·45	1·063	5·14
9	7·74	51·15	5·16	9·80	11·97	21·92	1·046	5·22
10	7·38	55·48	4·07	9·53	9·92	21·00	1·246	5·82
11	7·20	51·50	4·64	10·81	12·69	20·36	1·063	4·76
12	7·38	50·25	5·54	11·22	8·78	24·20	1·010	4·48
13	7·56	57·90	4·71	9·52	9·57	18·30	1·375	6·08
14	7·56	44·14	5·25	14·01	9·30	27·30	0·790	3·15
15	7·38	46·68	5·27	10·82	8·95	28·28	0·876	4·32
16	7·56	63·50	3·23	7·38	7·65	18·25	1·740	8·60
17	7·02	65·46	4·29	8·12	8·53	13·59	1·895	8·06
18	8·46	48·33	4·97	11·58	11·38	23·74	0·936	4·17
20	7·38	50·01	5·85	11·38	9·28	23·45	1·003	4·39
21	7·20	57·95	4·41	8·41	11·27	17·94	1·427	6·89
22	6·84	62·01	4·96	8·76	10·61	13·66	1·633	7·08
23	6·84	70·85	3·08	8·19	6·39	11·50	2·430	8·65
24	7·38	53·40	3·73	9·40	11·49	21·98	1·147	5·68
25	6·84	71·25	3·60	6·54	8·89	9·72	2·478	10·89
26	7·02	61·84	3·90	9·13	6·46	18·67	1·163	6·77
27	7·38	57·85	3·46	6·42	10·76	21·51	1·373	9·01

Case No.	Total Protein G/100 ml.	Protein Fractions					A/G Ratio	A/A2 Ratio
		A	α1	α2	β	γ		
28	7·20	63·57	3·37	8·50	7·63	16·93	1·745	7·48
29	7·38	53·18	4·28	8·69	11·24	22·61	1·136	6·12
30	6·30	59·45	4·04	8·50	9·71	18·30	1·466	7·00
32	6·66	70·60	3·14	6·88	8·72	10·63	2·400	10·26
33	6·84	55·40	3·80	10·23	7·49	23·08	1·242	5·42
35	6·48	65·10	3·01	7·72	8·47	15·70	1·865	8·43
36	7·20	58·31	4·29	8·71	9·96	18·73	1·400	6·69
37	6·30	69·50	2·86	5·92	7·44	14·28	2·280	11·74
38	7·74	48·94	5·37	12·63	12·26	20·80	0·959	3·87
39	6·30	64·00	3·87	8·34	10·74	13·05	1·780	7·67
40	7·20	62·17	3·25	8·22	7·32	19·04	1·640	7·56
41	7·38	72·71	2·99	6·06	7·92	10·32	2·660	12·00
42	6·84	73·80	2·63	4·55	7·59	11·44	2·740	16·22
45	7·02	72·43	2·89	6·35	9·04	9·29	2·630	11·40
46	7·02	57·40	3·70	8·10	10·20	20·60	1·350	7·09
47	8·82	57·61	3·19	5·91	14·29	19·00	1·360	9·75
48	7·20	61·01	2·35	8·37	8·57	19·69	1·560	7·29
49	7·74	46·58	3·08	9·26	13·02	28·06	0·872	5·03
50	7·38	56·19	3·86	6·69	8·13	25·14	1·283	8·40

severity of rheumatoid arthritis and severity of lung lesions. This question has been statistically examined on the basis of Table IV.

TABLE IV.
Distribution of Patients by Grades.

Rh. Arthritis Grade \ Lung Lesion Grade	0	1	2	3	4
4	1				3
3		1		5	4
2	1	1	2	5	5
1	2		1	2	
R		1	1	4	
0			2	2	2

In considering a table such as this one must bear in mind the factors governing selection of patients. In the present case those with Caplan's syndrome were often referred by other physicians and from the Pneumoconiosis Medical Panel. Those with characteristic lung lesions but without arthritis were similarly referred, but no special effort was made to locate and examine miners with rheumatoid arthritis alone. Thus there is an artificial relative deficiency in the first two columns of the table (those without specific lung lesions) and an absolute deficiency in the bottom left-hand cell (0 x 0) since no subjects were included in the series who had neither rheumatoid arthritis nor characteristic lung lesions. This cell might equally well contain a number representing all the South Wales miners <u>not</u> suffering from either of these

conditions.

For the purposes of statistical analysis the table has therefore been redrawn, omitting the first two columns and reducing the number of rheumatoid arthritis grades to the two used in deriving the "Rh. Ratio" of Fig. 6.

Table V shows the numbers re-arranged in this way, together with the numbers expected on the basis of complete independence.

TABLE V.

Distribution of Patients by Grades and Expected Distribution

Rh. Arthritis Grade \ Lung Lesion Grade	2		3		4		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Totals
2, 3 & 4	2	3·79	10	11·37	12	8·84	24
0, R & 1	4	2·21	8	6·63	2	5·16	14
Totals	6		18		14		38

A χ^2 test shows $p = 0{\cdot}055$ which, though just short of the conventional level of significance, is strongly suggestive of some association between the two factors.

Assuming there to be such an association, how does this affect the interpretation of Fig. 6? If rheumatoid arthritis and the lung lesions of Caplan's syndrome both produce the same kind of change in the serum protein pattern (e.g. reduction of the albumin/α2 globulin ratio) and if the two are positively associated so that severe lung lesions usually accompany severe arthritis it might be expected that this would exaggerate the differences in protein pattern between mild and severe grades of either condition. It is therefore all the more surprising that no clear trend is ap-

parent in Figure 6.

The possibility that the absence of obvious correlation between protein fraction levels and lung lesion grades might be due to selection was examined. The fact that patients with characteristic lung lesions were actively sought and persuaded to attend for investigation, even if not clinically ill, whereas no such effort was made to collect cases of rheumatoid arthritis without specific lung lesions, means that the latter were likely to be iller men; those who were seen must at least have been ill enough to attend the out-patient clinic of their own accord or on the advice of their doctor. The shape of the curves in Fig. 6 is consistent with this hypothesis since those without specific lung lesions (Grades 0 and 1) show greater abnormality in respect of each protein fraction than do those in Grade 2.

On the other hand, Grades 2, 3 and 4, where no bias due to selection applies, show for each protein fraction a trend toward increasing abnormality with increasing severity of lung lesions. These trends in Grades 2 - 4 have been analysed statistically and, as this analysis was rather complicated, a full account of it is included as Appendix A. The results are as follows:-

1. The mean values for the three grades show a significant trend in the case of Albumin ($p < 0{\cdot}001$) α1-globulin ($p < 0{\cdot}025$), α2-globulin ($p < 0{\cdot}001$), β-globulin ($p < 0{\cdot}05$), γ-globulin ($p < 0{\cdot}005$) and the albumin/α2-globulin ratio ($p < 0{\cdot}001$).

2. These trends may, however, consist of two components namely, an effect of rheumatoid arthritis and an effect of lung lesions. In an attempt to separate the latter, indi-

vidual regressions were calculated for each protein fraction within each rheumatoid grade. Comparison of these individual "within group" regression coefficients with each other showed no significant difference for any protein fraction. The weighted average "within group" regression was therefore taken as an index of lung lesion effect. The values were statistically significant in the case of albumin ($p < 0{\cdot}01$), α_2-globulin ($p < 0{\cdot}01$), γ-globulin ($p < 0{\cdot}05$) and the albumin/α_2-globulin ratio ($p < 0{\cdot}005$).

3. Comparison of the mean "within group" regressions with the slope of the means showed the latter to be always of the same sign and greater than the former. This difference represents the rheumatoid component and was significant in the case of albumin ($p < 0{\cdot}01$), α_2-globulin ($p < 0{\cdot}025$) and the albumin/α_2-globulin ratio ($p < 0{\cdot}01$). In the case of γ-globulin the difference was significant only at the 10% level.

From these results it seems justifiable to conclude that the lung lesions are associated with protein changes independent of those due to rheumatoid arthritis at least in the case of albumin, α_2-globulin, γ-globulin and the albumin/α_2-globulin ratio. Such protein changes are consistent with the tuberculous theory of their aetiology. Some of the tests are not very discriminating, and the absence of statistical significance in the case of other protein fractions does not imply that, with a larger sample, a difference would not be found.

One curious and possibly significant fact emerged from the statistical analysis. In the case of both α-globulins the within-group regression for the group

without rheumatoid arthritis (Grade 0) was of opposite sign to all the other within-group regressions, including that for the remission group. The reverse slope for α2-globulin was of sufficient magnitude to produce a similar effect in the albumin/α2-globulin ratio though the Grade 0 regression for albumin was of normal sign. It is tempting to speculate whether the failure of the individuals in this group to develop clinical rheumatoid arthritis could be associated with some abnormality in their α-globulins accounting also for the anomalous response of the latter to the presence of rheumatoid lung lesions. In this connection it is interesting to note (Table VI) how normal are the six members of this group in respect of E.S.R. (3 normal), A/G ratio (3 normal) and A/α2 ratio (4 normal). None of the six is abnormal by all three criteria and cases 41 and 42 are normal by all three although both had well developed, typical lung lesions the nature of which was in each case confirmed by a positive D.A.T.

TABLE VI

Results Obtained in Six Patients with Lung Lesions, but no Arthritis.

Series No.	Caplan Category	A/G	A/α2	E.S.R.	D.A.T.
17	2	1·895	8·06	3	< 4
23	2	2·430	8·65	19	256
28	4 C	1·745	7·48	24	< 4
41	3 Cal	2·660	12·00	9	128
42	3	2·740	16·22	5	32
47	4	1·360	9·75	36	128

C = Cavitation.

Cal = Calcification

Study of the protein patterns of 12 "normal" subjects revealed a considerable range of values but little overlap with the values obtained in the group of patients. The results are presented in Table VII. No reliable estimate of normal limits or even of a lower limit of normal can be derived from so small a group, but in the single case of the albumin/α2-globulin ratio an attempt has been made to do this approximately by comparing the values in Table VII with those in Table III.

If the lower limit of normal were set at 10, only one of the "normal" subjects appears abnormal, but 6 patients appear normal. Furthermore, "normal" subject number 10 should probably not have been so described as he had fairly recently undergone a block resection of axillary glands suspected of malignancy; no malignancy was found, but chronic inflammatory change of unknown aetiology was present If this subject is excluded the lower limit of normal could be placed as high as 12 without excluding any others, and at this level only two patients appear normal. This level, though convenient, is probably unrealistic since twelve (or eleven) normal subjects could not be expected to represent the entire range of normal.

The A/α2 ratios in Table VII are consistent with a normal distribution, the skewness being small (g_1 = 0·76 ± 2·09) and, assuming this distribution, it can be calculated that 95% of normal subjects would have A/α2 ratios above 9·24. This level includes all of the subjects in the "normal" group, including subject 10, and excludes all but 8 of the fortyfive patients. Of these eight all but two fall into arthritis grades 0, 1, and R, the remaining two

TABLE VII

Serum Protein Analysis of Twelve Normal

Case No.	Age	Protein G/100 ml.	A	α1	α2	
1	44	7·38	76·55	2·83	3·98	
2	45	7·02	74·33	3·56	4·51	
3	40	7·20	70·95	4·39	5·44	
4	45	7·56	72·00	3·81	4·89	
5	40	7·02	73·39	3·68	5·36	
6	44	6·84	78·67	1·62	3·19	
7	43	6·66	74·00	3·01	4·00	
8	42	7·20	75·92	2·27	4·45	
9	49	6·66	69·36	3·68	5·20	1
10	48	7·56	67·28	4·19	7·02	1
11	42	6·66	72·08	3·51	6·00	
12	43	6·48	75·18	3·03	4·46	
Total	525	84·24	879·71	39·58	58·50	9
Mean	43·75	7·02	73·31	3·30	4·88	
Mean Values of Gilliland et al (1956)		7·26	57·17	3·99	8·13	1
Mean Values of Gilliland et al after albumin correction			68·60	2·92	5·96	

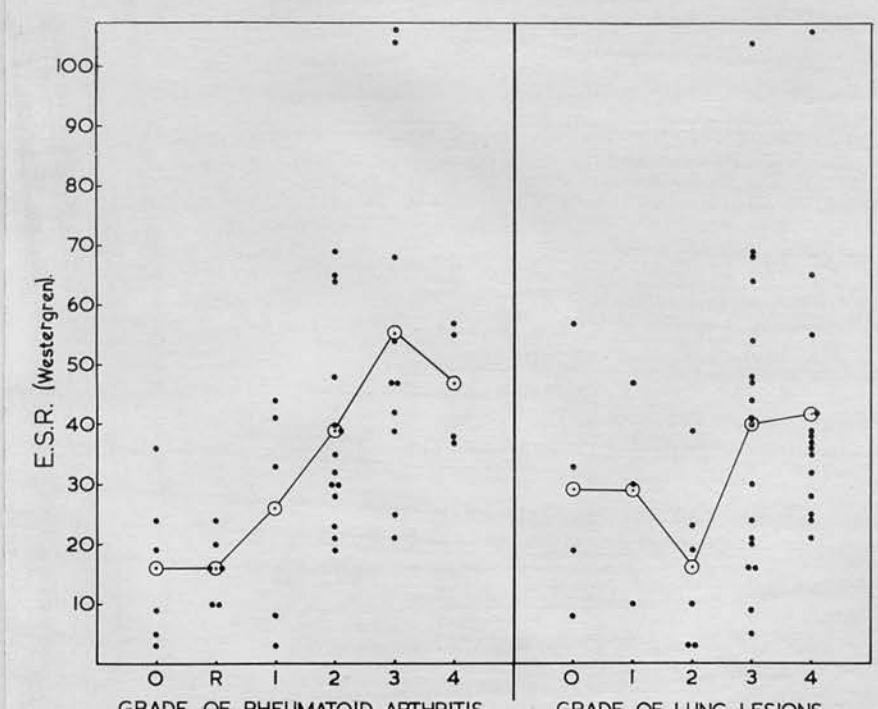
having grade 2 arthritis. Only three of them had an abnormal E.S.R. (above 10 mm. in 1 hour) including the two patients with grade 2 arthritis. This suggests that to set the lower limit of normal for the albumin/α2-globulin ratio at 9·24 would be as consistent with clinical observations as can be expected as well as being statistically acceptable, and this value was therefore arbitrarily selected.

The mean values for the twelve normal subjects are compared at the foot of Table VII with those for 28 normal adults published by Gilliland et al (1956). It is clear that striking differences exist, particularly between the albumin values. This is to be expected since the values published by Gilliland et al were derived by direct densitometry and it has been shown (Gorringe, 1957) that this method underestimates albumin. That albumin has been underestimated is confirmed by the very low A/G ratio given by Gilliland et al for their normal subjects.

The protein patterns of all the 45 patients in the present series (though not of all the twelve normal subjects) were analysed by planimetry of densitometer traces as well as by elution in order to derive if possible an albumin correction factor which could be used in conjunction with the former technique. It was found that no single factor could be successfully applied in every case, indeed the factors required to correct the albumin values in individual cases ranged from xI to nearly x2. The mean correction factor however, was x1·2 and this can legitimately be applied to the mean of a number of analyses. This has been done with the mean normal values published by Gilliland et al. It will be seen that this manoeuvre greatly reduces the differ-

FIGURE 7.

Scatter diagram relating the severity of arthritis (left) and lung lesions (right) to the E.S.R.



ences between the two series but does not eliminate them.

Comparison of Figure 4 with traces published by Gilliland et al reveals a difference which may explain part, if not all of the remaining discrepancy; the densitometer traces obtained in the present series approach closer to the base-line in the troughs between peaks, although that chosen as Figure 4 shows this feature to a less than average extent. This would have a relatively greater effect upon the low globulin peaks than upon the high albumin peaks, and so tend still further to reduce the A/G and A/α2 ratios in the series of Gilliland et al, compared with those in the present series.

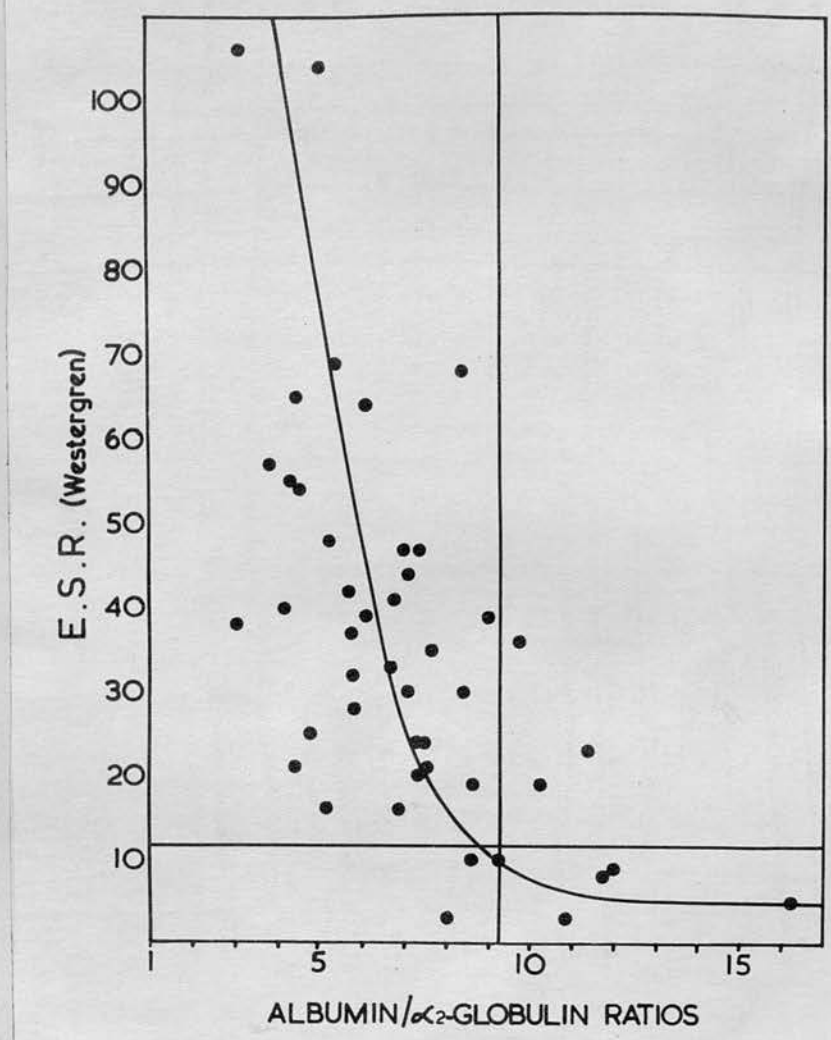
E. S. R.

When the E.S.R. values are plotted against the grades of rheumatoid arthritis (Fig. 7) the expected rising curve is found except that a fall occurs from Grade 3 to Grade 4. The probable explanation of this fall is that some of the Grade 4 cases, though severely disabled by the disease, are tending towards a "burnt-out" stage in which the E.S.R. falls. Alternatively the explanation may lie simply in the small number of cases in Grade 4. It is of interest that the mean E.S.R. of the Remission group is the same as that of the group without arthritis, whereas the serum proteins of the Remission group showed greater abnormalities than the group with Grade 1 active arthritis. This suggests that the serum protein pattern reverts to normal more slowly than the E.S.R. when remission of rheumatoid arthritis occurs.

Fig. 7 also shows the E.S.R. plotted against the

FIGURE 8.

Comparison of A/α2 ratio with E.S.R. The vertical and horizontal lines represent lower limit of normal for A/α2 and upper limit of normal for E.S.R. respectively.



severity of lung disease. The result is similar to that of the A/α2 ratio similarly plotted (Fig. 6) in that Caplan Grade 2 cases show the least abnormality in both. No doubt this also can be attributed to the method of selection tending to make lung-lesion Grades 0 and 1 an iller group than Grade 2.

Gilliland et al (1956) showed that the albumin/α2-globulin ratio was a better index of the severity of pulmonary tuberculosis than the E.S.R. since 50% of their cases had a normal E.S.R. whilst only 10% had a normal A/α2 ratio. In the present series the two are compared in Figure 8. The E.S.R. is normal in 7 cases (15·5%) and the A/α2 ratio in 8 (17·7%). It therefore appears that in Caplan's syndrome there is little to choose between the two though remission of rheumatoid arthritis is apparently associated with a more rapid return to normal of the E.S.R. than of the A/α2 ratio.

D. A. T.

The D.A.T. was positive (titre of 1/32 or greater after 18 hours) in 33 cases (73·4%). Among the 32 cases of Caplan's syndrome the corresponding figure was 84·4%. Four of the six cases without arthritis had a positive D.A.T. (66·6%) but only 2 (28·6%) of the 7 patients with arthritis but no definite lung lesions. Table VIII shows the distribution of positive and negative D.A.T.'s between those with and those without characteristic lung lesions. The difference is significant at the 1% level.

FIGURE 9.

Relationship between γ-globulin (above) and β-globulin (below) and the inverse of the D.A.T. titre.

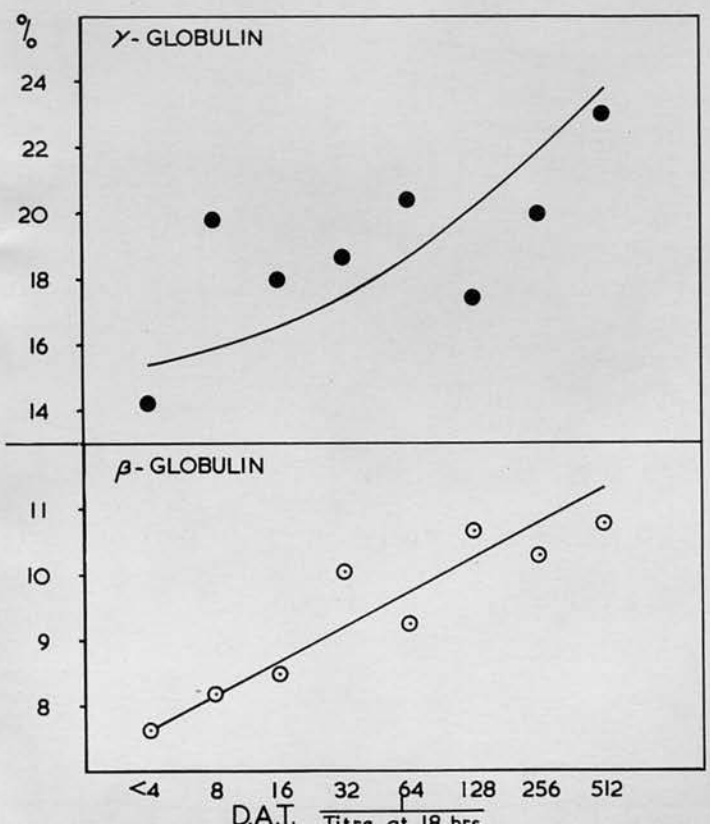


TABLE VIII

Distribution of Positive and Negative D.A.T. by Lung Lesion Grades

Lung Lesion Grade \ D.A.T.	Positive	Negative	Total
2 - 4	31	7	38
0 & 1	2	5	7
Total	33	12	45

These findings are consistent with those of Ball (1955) and the tendency to which he drew attention for the D.A.T. titre to increase with increasing severity of lung lesions was also apparent in the present series (Table IX).

TABLE IX

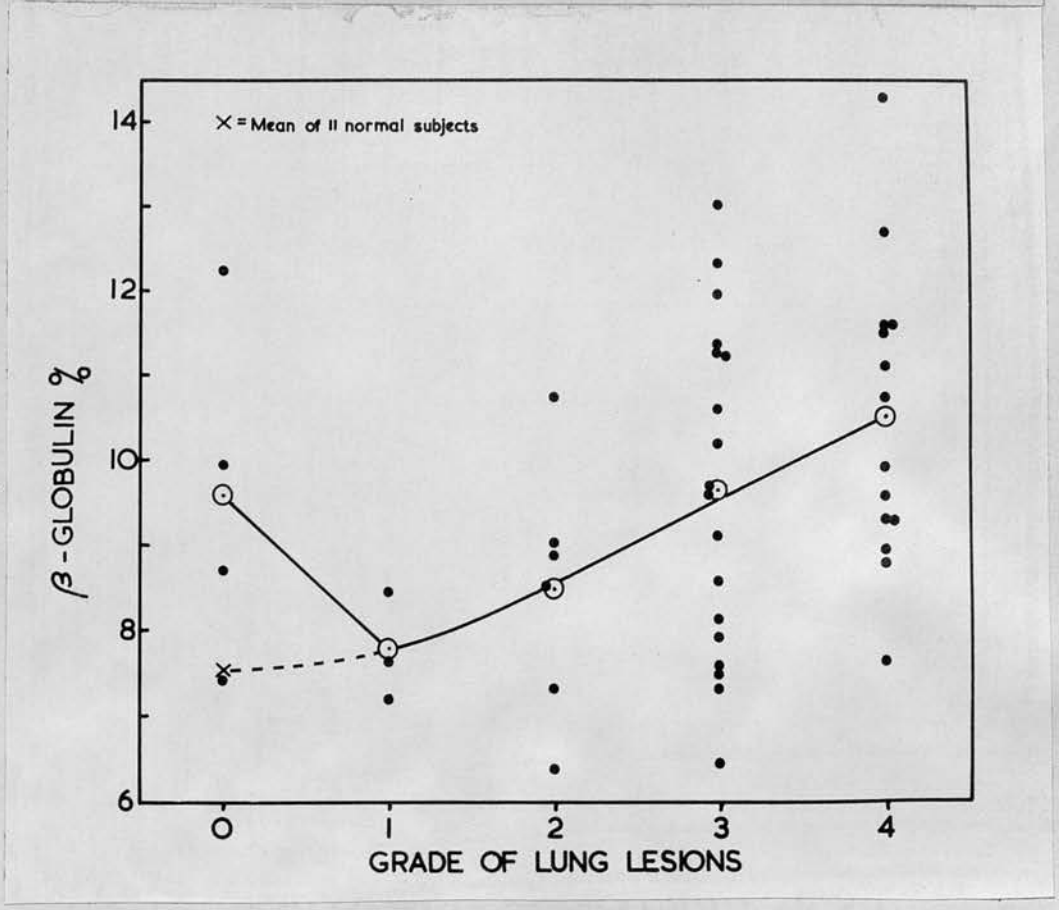
Proportion of Subjects with High D.A.T. Titre by Lung Lesion Grades.

Lung Lesion Grade	0	1	2	3	4
% of high titres (1/256 and above)	0	0	17	28	36

Though Franklin, Kunkel and Ward (1958) found the quantity of rheumatoid factor in the serum of their patients to correlate well with the γ-globulin level and indeed to account for a large part of the elevation of γ-globulin above normal, no correlation could be demonstrated in the present series between D.A.T. titres and the γ-globulin level ($p > 0{\cdot}1$). Instead there was a suggestion of a correlation with β-globulin (Figure 9) and this proved

FIGURE 10

Scatter diagram relating the severity of lung lesions to the β-globulin level.



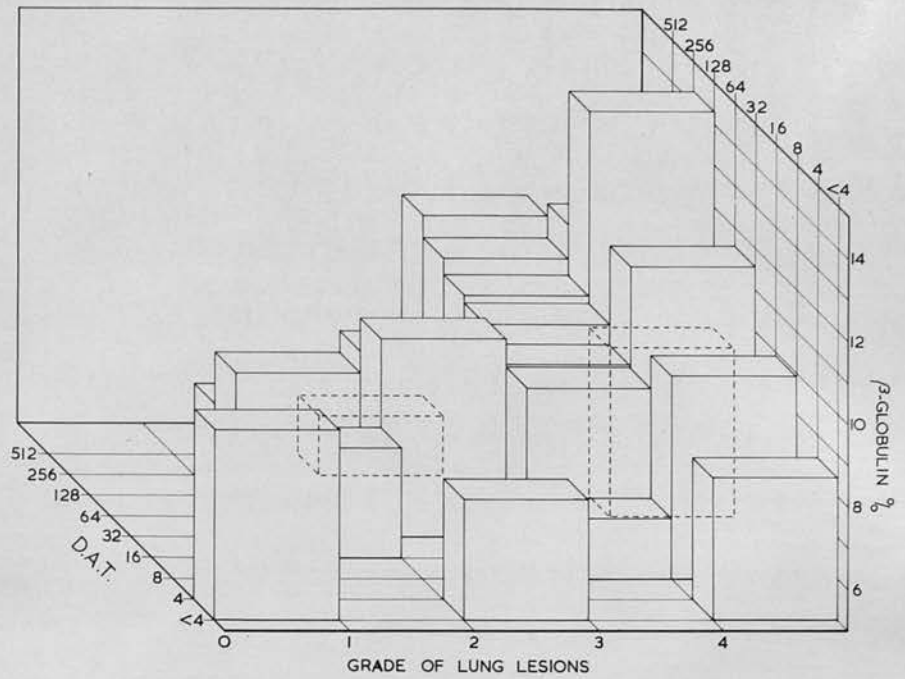
to be significant ($p = 0{\cdot}05$).

That the D.A.T. should be significantly correlated with both the lung lesion grade and with the β-globulin level is surprising in view of the absence of independent correlation between the two latter. Figure 10 shows the curve obtained when the β-globulin level is plotted against lung lesion grades and, if grades 0 and 1 are disregarded because of the bias introduced by selection into these groups (as was done in the analysis of other protein fractions), the trend from grade 2 to grade 4 is significant at the 5% level ($0{\cdot}05 > p > 0{\cdot}025$). This trend could be due in part to the effect of arthritis which has been shown to be on average more severe in those with severe lung lesions (Table V) and when the attempt was made statistically to separate the effects of the two no significant correlation could be demonstrated between lung lesions alone (weighted average within-group regression) and the β-globulin level. On the other hand, there is no significant correlation between the rheumatoid grade and the β-globulin level ($p > 0{\cdot}25$) so that the trend from Grade 2 to Grade 4 in Figure 10 may be acceptable at its face value even though this could not be demonstrated statistically.

Accepting for the moment that Figure 10 shows a true relationship, an interesting three-fold association emerges between severity of lung lesions, D.A.T. titre and β-globulin level. An attempt has been made in Figure 11 to show this relationship in the form of a three-dimensional histogram. The height of each block represents the mean β-globulin level of the subjects in the group defined by their lung lesion and D.A.T status. Though

FIGURE 11.

Three-dimensional histogram relating lung lesion grades and D.A.T. titres to the β-globulin level.



the numbers represented by each block are small and those at the left and bottom of the diagram are subject to the same selection bias as has been mentioned before, there remains a strong suggestion of a rising trend from bottom left to top right of the diagram. It is hoped to show that there is nothing improbable about such an association.

RHEUMATOID NODULES

Definite subcutaneous rheumatoid nodules were present in 16 (41%) of the 39 cases with arthritis and in none of those without it. This rather high incidence probably reflects the care taken to search for nodules and the relatively strict criteria used in the diagnosis of rheumatoid arthritis, rather than any increased tendency for Welsh miners to develop subcutaneous nodules.

Of these 16 cases the D.A.T. was positive in all but one which is consistent with the observation of other workers that the test is rarely negative in cases with rheumatoid nodules. (Ball, 1952, Jacobson, Kamerer, Wolf, Epstein and Heller, 1956, Kellgren & Ball, 1959).

Only one subject (Case 36) with subcutaneous nodules was without nodular lung lesions, and in this case the joint symptoms and nodules had been present for only 2 days. This was also the only case with a negative D.A.T.

In view of the well-known association between subcutaneous nodules and a positive (often strongly positive) D.A.T., an association was sought between the presence of nodules and changes in the various protein fractions. The 39 subjects with rheumatoid arthritis were divided into two groups, one consisting of the 16 with subcutaneous nodules

plus two in whom such lesions had previously been present and the other of 21 subjects who were not known ever to have had subcutaneous nodules. In the case of γ-globulin the difference was significant only at the 10% level and there was not even a suggestion of significance in respect of any other protein fraction or even the albumin/α2-globulin ratio ($0{\cdot}5 > p > 0{\cdot}2$).

PSORIASIS AND OTHER SKIN DISEASES

Six of the 45 patients had definite psoriasis (13·3%) compared with 3 cases of allergic skin disorders. There was one case each of familial ichthyosis and of herpes zoster. The incidence of psoriasis is very high compared with that usually reported. For example Ball (1952) found only 6 out of 178 males with rheumatoid arthritis to have psoriasis and Gribble, (1955) reported 4·2% in his own series and quoted values between 2·6 and 4·6% from 6 other series.

Among the 6 males and 8 females similarly affected in the series reported by Ball (1952) only one case had a positive D.A.T. whereas 4 of the 6 cases with psoriasis in the present series had positive D.A.T.s. All the 4 positive cases had characteristic rheumatoid lung lesions and the two negative cases did not.

FAMILY HISTORY

Seven of the 45 patients gave positive family histories of rheumatoid arthritis. In 6 cases (13·3%) parents and/or siblings were affected, which agrees with the incidence reported by Miall (1955). The seventh positive history referred to a maternal grandmother. All but one of the seven cases suffered themselves from arthritis. Only

4 of the cases in the present series were included in the series reported by Miall (1955). One of these had a positive family history.

AGE OF ONSET

It was already known (Miall et al, 1955) that either the lung lesions or the arthritis may develop many years before the other. In the present series an effort has been made to determine the age of onset of each. In the case of arthritis this has usually been simple as a patient can generally give a fairly accurate estimate of when it first developed. In the case of lung lesions it has rarely been possible by examination of old radiographs to date the onset within a year and in most cases only a minimum duration can be arrived at because the characteristic lesions were already present at the date of the first available radiograph.

In spite of this limitation it is possible to say (Table X) that the lung lesions certainly developed first in 29 cases and the onset of arthritis definitely preceded them in only 6 cases; in 4 of these, lung lesions remained absent at the time of examination. In the remaining 10 doubtful cases there is a strong probability that the arthritis developed first in only one case. In the remainder there is no evidence either way. Even if we assume that the arthritis preceded the lung lesions in all the doubtful cases, the latter developed first nearly twice as often.

TABLE X

No.	Age Years	Years since Onset of Lung Lesions	Years since Onset of Rh. A.	Age at Onset of Lung Lesions	Age at Onset of Rh. A.
1	35	1/12	9/12	35	34
2	61	2 +	8/12	59 -	60
3	56	2 +	1½	54 -	54
4	55	15	4 9/12	40	50
5	28	9	7	19	21
6	42	10	3	32	39
7	53	11 +	15	42 -	38
8	53	3 +	2 7/12	50 -	50
9	43	5 +	5	38 -	38
10	42	6 +	3 5/12	36 -	39
11	47	3	6	44	41
12	34	1 +	1	33 -	33
13	43	2 +	2 3/12	42 -	41
14	53	5 +	1 10/12	48 -	51
15	46	5 +	5	41 -	41
16	46	3 +	3 6/12	43 -	42
17	40	7 +		33 -	
18	39	9 +	2	30 -	37
20	47	5 +	10/12	42 -	46
21	49	5 +	6	44 -	43
22	59	3 +	26	56 -	33
23	57	5 +		52 -	
24	43	10 +	15	33 -	28
25	44	2 +	8	42 -	36
26	58	1	3/52	57	58
27	66	4 +	3	62 -	63
28	43	7 +		36 -	

No.	Age Years	Years since Onset of Lung Lesions	Years since Onset of Rh. A.	Age at Onset of Lung Lesions	Age at Onset of Rh. A.
29	59	3 +	3	56 -	56
30	49	4 +	4	45 -	45
32	59		2		57
33	63	7 +	3	56 -	60
35	63	8 +	14	55 -	49
36	64		2 days		64
37	50		3½		46
38	33		5		28
39	45	8 +	6 9/12	37 -	38
40	44	6 +	6 10/12	38 -	37
41	58	2 +		56 -	
42	51	4 +		47 -	
45	34	6/12 +	5	33 -	29
46	50	7 +	1	43 -	49
47	61	9 +		52 -	
48	36	4 +	10	32 -	26
49	51	10 +	9	41 -	42
50	60	3 +	3	57 -	57

D I S C U S S I O N

At the time this investigation was undertaken (1955) the objective evaluation of rheumatoid activity had not been sufficiently developed to inspire confidence. The condition could be diagnosed with reasonable certainty, particularly in males, its severity could be assessed in terms of disability but most people would hesitate to express any quantitative opinion as to the degree of activity in a particular case. It was therefore decided to attempt only what could be achieved; after the condition had been diagnosed clinically and the diagnosis confirmed serologically and/or radiologically where possible, a case was designated active or inactive - i.e. in remission. This limitation was all the more necessary because several objective criteria such as the E.S.R., haemoglobin level, white count and temperature, could not be used as indices of activity in case they were influenced by the pulmonary pathology which was a feature of most cases.

This decision now appears timid, but it is gratifying to find that the criteria used to make the decision between activity and remission include all those recommended by Lansbury (1958) for the evaluation of rheumatoid activity except the E.S.R. This statement requires some qualification; the symptoms and signs used in the present study (see p.14) are not stated in quite the same way as Lansbury states them, but, if his "joint dysfunction" and "muscle weakness" can be considered to be included in the term "functional disability not attributable to residual deformity" and if "swelling" in the present study, together with "hot, red joints and intra-articular effusions" are acceptable as

indicative of "total amount of joint inflammation" then the statement is justified. That criteria additional to those found useful by Lansbury have been included, namely, rheumatoid nodules, hyperhidrosis and palmar erythema, is because the necessary exclusion of the E.S.R. was a considerable handicap and it was felt necessary to substitute for this valuable index as many as possible of the clinical features known to be associated with activity.

It is not claimed that the criteria used in allocating patients to the active or remission groups were ideal but they were the best that could be devised at the time. The identical mean E.S.R. level of the remission group and the group without arthritis suggests that the method worked well enough.

The design of the trial might more justifiably be criticised on the grounds that the method of selection resulted in those subjects without characteristic pulmonary lesions (i.e. with rheumatoid arthritis alone) not being comparable with those having these lesions. Such selection defects are difficult to avoid when dealing simultaneously with a common and a rare condition. In order to collect adequate numbers of the latter considerable efforts must be made to locate and examine cases, whereas patients suffering from a common disease such as rheumatoid arthritis present themselves in adequate numbers without any effort being made. In the present series it was realised too late that those coming of their own accord to an out-patient clinic are more likely to be clinically ill than those who have been persuaded to attend because of a reported radiological

abnormality. Fortunately, the number of patients without characteristic lung lesions was small in this series so that to exclude them from certain statistical analyses did not greatly reduce the number available for such analysis. It did, however, have the effect of amputating the tail of all curves relating lung lesion grades to other parameters so that various grades of severity could be compared only with each other and not with a zero grade as was possible in the case of rheumatoid arthritis. This is a severe defect and one which should have been foreseen and guarded against.

A third cause for retrospective regret is that serum electrophoresis was carried out by a method which did not separate the β1- and β2-globulins. The technique described by Laurell, Laurell and Skoog (1956) was not known to the author until the present study was almost completed but, had this method been used, it seems probable that more convincing evidence of an association between a β-globulin (probably β2) and the nodular lung lesions of Caplan's syndrome might have been obtained.

For the purposes of this discussion it will be assumed that the trend towards higher total β-globulin levels with increasing severity of lung lesions shown in Figure 10 is a true relationship and it has been shown that the slope from lung lesion grade 2 to grade 4 is statistically significant (see p.22); only when an attempt was made to show that the effect could be attributed to the pulmonary pathology independently of the rheumatoid arthritis did the results fail to achieve significance. The statistical method was admittedly not very discriminating and

the numbers were small so that failure to achieve significance is quite compatible with a real relationship and certainly there is no evidence that the effect was due to the rheumatoid arthritis. Kuhns et al (1955) asserted that the β-globulin level was sometimes elevated in rheumatoid arthritis but their electrophoretic method did not permit quantitative analysis so that this assertion was based solely upon inspection of the stained strips. Salt (1956) found the β-globulin elevated in five of twentysix patients, but his series included cases of inactive rheumatoid arthritis, osteoarthritis and fibrositis without any indication being given as to which diagnostic category the cases with high β-globulin belonged to. In the series reported by Ropes, et al (1954) elevation of β-globulin was apparent in only one patient, during periods of rheumatoid activity, although these workers used free electrophoresis which tends to accentuate β-globulin since its polysaccharide and lipid constituents contribute to the refractive index as well as the protein itself. On the other hand, Barhad et al (1956) claimed that the β-globulin of miners with pneumoconiosis tended to be increased whilst they were at work, though normal during hospitalisation, Pernis and Calo (1956) showed marked increase of mucoprotein in the serum of patients with silicosis which probably implies an elevated β-globulin level and Rosenkranz (1957) reported moderate elevations of β-globulin in both silicosis and silico-tuberculosis. The series of non-pneumoconiotic tuberculosis patients reported by Gilliland et al (1956) showed no correlation between the β-globulin level and the radiological

grade. If the apparent relationship between "Caplan lesions" in the lungs and elevation of the serum β-globulin is indeed a real one, the condition differs in this respect from tuberculosis.

It is generally assumed that the "Rheumatoid Factor" (R.F.) responsible for the agglutination of sensitised sheep erythrocytes is to be found in the γ-globulin fraction and as early as 1955 Svartz and Schlossmann claimed to have demonstrated this experimentally; yet in the present series the D.A.T. titre showed significant correlation with the β-globulin level but not with the γ-globulin. Examination of the literature revealed that in 1957 there were two quite distinct schools of thought on this subject; Svartz and Schlossman (1954) showed the agglutinating activity of rheumatoid serum to be contained in a globulin fraction which could be precipitated by diluting serum with 14 volumes of ice-cold distilled water, standing for 48 hours at 4°C and then centrifuging for 90 minutes at the same temperature. The following year the same authors (Svartz et al, 1955) published electrophoretic evidence that the cold-precipitable protein consisted mainly of γ-globulin but contained also β- and some α-globulin; the agglutinating activity peak corresponded exactly with the γ-globulin peak. The same year Lamont-Havers (1955), using a somewhat similar method of precipitation with cold distilled water but subsequently purifying his precipitate further by redissolving it in 15% saline, dialising against 0·85% saline and removing the fresh precipitate by further centrifugation, produced an active fraction which consisted of electrophoretically pure

γ-globulin. Franklin, Holman, Müller-Eberhardt and Kunkel (1957) demonstrated an abnormal 22s component in rheumatoid serum. It occurred in the faster-migrating end of the γ-globulin fraction as also did the agglutinating and γ-globulin-precipitating activity. Heated γ-globulin could completely absorb this 22s component leaving the supernatant serum devoid of precipitating or agglutinating activity. Franklin, Kunkel, Müller-Eberhardt & Holman (1957) showed later the same year that the 22s component could be dissociated into a 19s and 7s component of which the former was active in agglutination and F.II precipitation tests.

Rose, Ragan, Pearce and Lipman (1948) have been misquoted (Glynn, Holborrow and Johnson, 1957) as attributing agglutinating activity to β-globulin. In fact, they attributed it only to a fraction consisting of β- and γ-globulin. Wager and Alameri (1953), using the fractionation method described by Deutsch and Alberty (1947) found fraction C2 to be the most active; this consisted mainly (90%) of β-globulin. Robinson, Stuhlberg and Kuyper (1954) using salting out and dialysis techniques found the agglutinating activity in fractions precipitated between 9% and 14% Na_2SO_4 or between ionic strengths 0·06 and 0·04; the electrophoretic fraction "most consistently present in the concentrate" was β-globulin. Heller, Kolodny, Lepov, Jacobson, Rivera and Marks (1955) using a combination of the method of Deutsch, Gosting, Alberty and Williams (1946) with Cohn's method 6 (Cohn, Strong, Hughes, Mulford, Ashworth, Melin and Taylor, (1946) found all the activity in fraction III which contained 73·1% β- and only 7·4% γ-globulin; other Cohn-Deutsch fractions containing more

γ- or α-globulin were inactive and these authors therefore attributed the activity to β-lipo-protein. Thulin (1955), using paper electrophoresis showed the agglutinating activity to be spread over the faster moving γ-globulins and the whole of the β-globulin. Clark, Smyth & Haiby (1957) found agglutinating activity in Cohn fraction III (they did not state by which of Cohn's several methods this was prepared, neither did they characterise it electrophoretically, but it will certainly have contained a high proportion of β-globulin). When this fraction was absorbed with sensitised sheep cell stroma the activity was lost together with some protein-bound carbohydrate.

Ziff (1957) attempted to reconcile the discrepant findings of the workers quoted above by the suggestion "that in whole serum the rheumatoid factor interacts with faster moving proteins which raise its mobility, while in fractions containing only gamma globulin it remains with this fraction". This explanation does not fit the facts for among the protagonists of γ-globulin Lamont-Havers (1955) alone claims to have used a fraction "containing only gamma globulin", whereas of the β-globulin school only Thulin used "whole serum". The fraction used by Wager et al (1953) was probably more nearly pure β-globulin than Svarts and Schlossman's (1955) fraction was pure γ-globulin.

A more probable explanation of the paradox is suggested by the work of Laurell et al (1956). These workers observed that storage of serum in the liquid state even at 4°C resulted in a diminishing level of β2-globulin with a corresponding increase in γ-globulin; this was apparent after

as little as 2 days storage. No such shift of β to γ occurred when serum was stored frozen solid. In the present study all serum specimens were placed in the deep freeze immediately after separation and remained there frozen solid until used.

Few, if any, of the authors quoted above describe how their serum specimens were stored, but among those who favour the belief that R.F. is a γ-globulin the method of preparing the fractions studied involved prolonged exposure of the serum to temperatures well above its freezing point. It is probable that this allowed time for much if not all of the R.F. to change from a β2- into a γ-globulin; the methods of separation may even have contributed towards this change for it is noticeable that water-dilution methods were not used by any of the workers of the β-globulin school. The electrophoretic constitution of the euglobin fraction used by Ziff, Brown, Lospalluto, Badin and McEwen (1956) does not appear to have been published but its preparation alone occupies 48 hours and the demonstration by Lospalluto and Ziff (1956) that its active constituent was a γ-globulin involved cellulose ion exchange chromatography which must have involved its remaining longer still in the liquid state. In this connection it is relevant that Franklin et al (1958) and Kunkel, Simon and Fudenberg (1958) who estimated euglobulin by a turbidimetric method occupying only 30 minutes, found that the quantity of this fraction correlated better with the results of agglutination tests than did the quantity of γ-globulin. Moreover, Shetlar, Payne, Padron, Felton and Ishmael (1956) who used a euglobulin fraction prepared by

by the method of Ziff et al (1956) found a negative correlation between the haemagglutination titre obtained with it and the γ-globulin content of the fresh serum. This suggests that the R.F. was not contained in the γ-globulin fraction before the serum it was subjected to the procedures involved in the preparation of euglobulin.

Most recent studies have supported the γ-globulin school, but this is hardly surprising since the increasing refinement and complexity of the techniques employed will inevitably have involved more prolonged manipulation of liquid serum, permitting ample time for the β2- to γ-globulin transformation to take place. The methods used ranged from the simple but quite lengthy continuous flow electrophoresis employed by Rantz, Randall and Kettner (1959) who decided that R.F. was a fast-moving γ-globulin to the five-stage separations used by Williams, Steward, and Jenkins (1958) and by Heimer, Federico and Freyberg (1958). The latter produced a final product with more than 550 times the specific activity of the parent serum and apparently identical with the material isolated by Lospalluto et al, (1956). This was said to be a slow γ-globulin which, after the treatment it had received, was perhaps not surprising. Even such prominent protagonists of the γ-globulin school as Franklin et al (1958) have shown that, when agglutinating activity is sought in fractions separated by simple starch block electrophoresis, activity extends from the γ-globulin almost to the β-globulin peak and they admit that the normal 19s component was absent from electrophoretically separated γ-globulin, being replaced by a 22s peak, though the 19s

* ADDENDUM

Heimer, Schwartz and Freyburg (1960) reported the case of a patient with hypogammaglobulinaemia and arthritis who had exceptionally high sheep cell and latex agglutination titres (1:3,500 and 1:112,000 respectively). On starch block electrophoresis the active material was mainly β-globulin and the 19·7s macroglobulin eluted from sensitised sheep cell stroma "migrated at a significantly higher rate than human γ-globulin".

Heimer, R., Schwartz, E.R., and Freyburg, R.H.

Arth. & Rheum. 1960 (June) 3, 274.

component had been demonstrated in the ultracentrifugal pattern of the whole serum.

That agglutination reactions are usually negative in agammaglobulinaemic patients with arthritis might be regarded as supporting the γ-globulin character of R.F. but McEwen (1958) has cast doubt upon the identification of this condition with rheumatoid arthritis. He also points out that many of the patients are children in whom positive agglutination reactions are less common than in adult rheumatoid arthritis (Ziff, 1957., Kellgren et al 1959). Finally, one of the 6 arthritic subjects with agammaglobulinaemia reported by Vaughan and Good (1958) agglutinated sensitised sheep cells and sensitised human cells at a titre of 1:16 although his gamma globulin level was the lowest in the series, namely 3 mg.% by the sensitive immuno-chemical method. *

If R.F. is a γ-globulin one would expect it to be contained in Cohn Fraction II. This fraction of normal serum is used in many of the precipitation and agglutination tests as a source of "Reactant", but no test has been devised which uses F.II of rheumatoid serum alone; indeed, there are modifications of the standard tests in use which dispense with this fraction (Singer and Plotz, 1958) using only a euglobulin fraction of the patient's own serum as the source of both R.F. and Reactant. The high percentage of positive reactions obtained by Singer et al (1958) using this test, compared with other methods in which F.II was used, suggests that F.II of normal serum must be inhibitory, and there is ample other evidence for this (Epstein & Ragan ,1956, Ziff et al, 1956, Ziff,1957, Rantz et al,1959). That the F.II

content of rheumatoid euglobulin does not cause inhibition has been explained as due to masking by the presence of excess R.F. (Ziff, Schmid, Lewis & Tanner, 1958).

Enough has been said to justify accepting the observed correlation between β-globulin levels and the D.A.T. titre and, though based perhaps upon less convincing evidence, a probable relationship between β-globulin and the severity of "Caplan lesions" has also been suggested. A relationship between these lesions and the D.A.T. titre has been previously demonstrated (Ball, 1955) and is confirmed in the present study. This three-fold relationship, which is presented diagrammatically in Figure 11 is particularly interesting in the light of the work by Pernis and others on the hyaline tissue of silicotic lesions. Pernis and Calo (1956) showed a highly significant increase of serum mucoproteins in silicosis and attributed this to increased fibroblastic activity. On the assumption that these mucoproteins were, more directly, derived from the ground substance of connective tissue (Pirani and Catchpole, 1951) Pernis and Ghislandi (1956) analysed the non-collagenous protein fraction of silicotic hyaline tissue by quantitative paper chromatography of hydrolysates. They showed that its amino-acid constitution corresponded with that of serum β-globulin and Pernis, Bairati and Frigerio (1956) later confirmed the identification of β-globulin by x-ray diffraction analysis and by electrophoresis. It is particularly interesting that the total carbohydrate and hexosamine content of "Fraction I" prepared by Pernis and Clerici (1957) agrees closely with that found in a similar fraction obtained from the fibrinoid material of rheumatoid nodules (Consden, Glynn and Stanier, [1953]).

Svartz and Schlossmann claimed as early as 1954 to have produced a haemagglutinating substance resembling the rheumatoid factor by the action of bacterial enzymes on human and bovine collagen tissue. Complete identity of this substance with the rheumatoid factor is still unproven (Svartz, 1956) but no differences have as yet been demonstrated. If the naturally-occurring rheumatoid factor is also produced by the action of enzymes on collagenous tissue, that occurring as lung nodules in Caplan's syndrome is obviously favourably placed from the point of view of accessibility to the bacteria, and that the lesions have at some stage contained bacteria is proved by the presence in them of D.A.P. (Consden et al, 1955 and 1957) This may be the explanation of the association between "Caplan lesions" in the lungs and a positive D.A.T. - even in the absence of arthritis.

Kunkel (1958) stated: "an exploration of the processes leading to the production of the rheumatoid factors should be a rewarding approach for further investigation". The present author no longer has facilities for such investigation, but the hypothesis which follows is consistent with what is already known about the R.F. and susceptible of experimental confirmation at a number of points. It is to be hoped that other workers will be encouraged to undertake appropriate experiments.

Kunkel (1958) considered the possibility that R.F. might play a causative role in the pathogenesis of rheumatoid arthritis but concluded that there was no evidence to support this. Vaughan (1959) transfused high-titre rheumatoid plasma into volunteers and was unable to

show any harmful effect. Numerous workers, however, have emphasised the relationship between positive agglutination reactions and some of the secondary features of rheumatoid disease (Sokoloff and Bunim, 1957, Kunkel, 1958, Christian, 1959, Epstein and Engleman, 1959, Kellgren et al, 1959) and in the case of subcutaneous nodules (Kunkel, 1958, Kellgren et al, 1959) and "Caplan Lesions" (Caplan, 1959) it has been suggested that R.F. may indeed play a causal part.

The mechanism proposed is spontaneous precipitation of R.F.-γ-globulin complexes in small arterioles followed by inflammation and necrosis. This sounds reasonable since precipitation of this kind can be demonstrated in vitro when high titres of R.F. activity are present (Epstein, Johnson and Ragan, 1956, Epstein, Engleman and Ross, 1957, Mannik, Brine & Clark, 1958) and in the case of Caplan's syndrome minute particles of mineral dust might facilitate precipitation as bentonite does in vitro (Bloch & Bunim 1959). But the lesions attributed to this mechanism can occur in the absence of demonstrable R.F.

Subcutaneous rheumatoid nodules are usually associated with positive agglutination reactions, but in few large series is the association demonstrable in 100% of cases. Kellgren et al (1959) found the sheep-cell agglutination reaction positive in 100% of their male patients with nodules but in only 92% of the females. Jacobson, et al (1956) obtained 95% positive sheep cell and 92% positive latex agglutination reactions. Caplan, Cowen and Gough (1958) found the Rose test negative in a patient with both subcutaneous and pulmonary nodules. In the present series

one of the 16 patients with subcutaneous nodules (6·7%) and 7 of the 32 patients with pulmonary nodules (21·9%) had a negative D.A.T. It might be argued that in the few cases showing negative agglutination tests positive results would have been obtained had the test been done while the nodules were developing. An opportunity arose to do this during the present study.

Case 36, who was admitted to hospital for treatment of chronic bronchitis associated with simple pneumoconiosis, began complaining of pains in several joints, especially those of the hands and arms, while he was in the ward. Small, soft nodules were present on both elbows and the patient was certain these had not been there previously. The nodules increased in size during the following week, though joint pains were controlled with small doses of aspirin, and one was then excised for histological examination. The pathologist confirmed our suspicion that the lesion was a rheumatoid nodule yet the D.A.T. performed upon serum collected when the joint symptoms and nodules had been present for only two days was completely negative. The test was repeated after the nature of the lesions had been confirmed histologically but remained negative. Nodular pulmonary lesions were absent at the time arthritis and subcutaneous nodules developed and plans were made to follow this patient's course in the hope of observing and perhaps treating the earliest stages in the development of "Caplan lesions". Unfortunately, the patient died of myocardial infarction three months later. At that time the lungs still contained no characteristic nodules. If R.F. plays a causative role in the development of subcu-

taneous nodules, it is obviously not the only possible cause.

Cases of Caplan's syndrome in whom fresh pulmonary lesions were just developing were also included in the series though no patient developed his first lesion while under observation. It was found, however that the D.A.T. was most often positive and the titre highest in patients whose pulmonary lesions were fully developed and often of long standing as indicated by old radiographs and by confluence of lesions.

Kunkel (1958) suggested that the splenomegaly and lymphadenopathy of Felty's syndrome may be due to hypertrophy of types of cells producing R.F. These cell types have been identified by Mellors, Heimer, Corcos and Korngold (1959) as plasma cells and the reticulum cells of the germinal centres of some lymphoid follicles. Using R.F.-specific fluorescent antibody they identified these cells also in synovial membrane and subcutaneous nodules. Gough et al (1955) described similar cells in the peripheral zones of Caplan lesions particularly in the lumen of small blood vessels. They did not lay much emphasis on these cells, the significance of which was not at that time suspected but Seal (personal communication) has since confirmed that plasma cells are present "in large numbers". Sokoloff et al (1957) described the predominant cell type in the adventitial inflammatory reaction associated with some cases of rheumatoid arteritis as "large mononuclear" and specifically-mentioned plasma cells in several cases.

It is now suggested that subcutaneous rheumatoid nodules, "Caplan lesions", and perhaps some other systemic rheumatoid lesions, so far from being caused by precipita-

tion of R.F.-γ-globulin complexes, may rather be sites of synthesis of the rheumatoid factor. [The two are not mutually exclusive, and both could occur, thus setting up a vicious circle and accounting for the very high agglutination titres often observed in the presence of such lesions]. The presence of appropriate cell types has been demonstrated in several tissues, but only in lymph nodes, synovial membrane and subcutaneous nodules have they been shown to contain R.F. (Mellors et al, 1959).

Svartz et al (1954) produced a substance resembling R.F. from collagen, Pernis et al (1956 - 1957) have shown the ground substance of some collagenous hyaline tissue to consist of β-glycoprotein and the experiments of Clark et al (1957) strongly suggest that R.F.is also a β-glycoprotein, It is therefore further suggested that the cells responsible for producing R.F. use degenerating collagenous tissue as their raw material That the "Fraction I" prepared by Pernis et al (1957) from silicotic hyaline tissue resembled that derived by Consden et al (1953) from the "fibrinoid" of rheumatoid nodules is consistent with this hypothesis. The hypothesis is also consistent with the apparent association observed during the present study between the D.A.T. titre, the severity of Caplan-type lung lesions and the level of serum β-globulin.

The small piece of research reported in this thesis is incomplete inasmuch as its implications have not been followed up but in this sense most research is incomplete, since one thing inevitably leads to another and it must be rare for any one individual to follow a lead to its ultimate conclusion. The last section of this dis-

cussion consists of some suggestions for experimental work which, if done, would confirm or refute the hypothesis proposed regarding the processes leading to the production of rheumatoid factor.

First it is necessary to confirm that in fresh serum R.F. occurs as a β-glycoprotein with a sedimentation constant of about 19s and that the 22s γ-globulin reported by so many workersis in truth an artefact. This requires separation by the quickest and least traumatic method available which is probably starch-block electrophoresis at a temperature only just above freezing point of the buffer.

Secondly, the fraction isolated in this way could be further purified by adsorption of the active constituent onto sensitised sheep cell stroma and subsequent elution (Heimer, Federico, Schwartz and Freyberg, 1959). The material isolated in this way would be available for further characterisation by the chemical and physical methods used by Pernis et al (1956-57) and comparison of the results obtained with the observations made by these workers upon β-glycoprotein derived from collagenous hyaline tissue might permit a conclusion to be reached as to whether R.F. could have been derived from such a source.

Thirdly, it must be considered whether the demonstration of R.F. in plasma and reticulum cells (Mellors et al, 1959) necessarily implies synthesis by these cells. The alternative explanation would be that such cells absorb circulating R.F. in which case the infusion (particularly the local infusion) of high-titre rheumatoid serum or euglobulin might be expected to enhance the reaction of frozen sections of perfused tissue with R.F.specific

fluorescent antibody. This experiment might be done either in vivo or in vitro.

Fourthly, fluorescent antibody staining methods applied to frozen sections of synovial membrane, subcutaneous or Caplan nodules excised from individuals with persistently negative agglutination reactions, would be informative; the absence of fluorescent plasma cells would be consistent with the hypothesis that R.F. originates in such cells but, should their presence be demonstrated, further investigation of the serum for inhibitory activity would be indicated.

Fifthly, fluorescent antibody staining methods might be applied to appropriate tissues excised from patients suffering from other conditions associated with positive agglutination reactions including systemic lupus erythematosus, sarcoidosis, syphilis, hepatic cirrhosis and kala azar.

Sixthly, cases of spontaneous abortion associated with positive agglutination reactions (Gray, Tupper and Rowse, 1958) would be particularly worthy of investigation since here R.F. or a similarly reacting substance is associated with fibrinoid necrosis of placental villi and also with the high β-globulin levels characteristic of pregnancy (Brown, McCandy, Gillie and Doyle, 1959) and particularly of abortion (Langman, van Drunen and Bouman, 1959).

Finally, it is conceivable that the production of R.F. from certain cell types could be demonstrated in tissue culture, in which case the raw materials used in its synthesis might be identified by radio-active tracer-studies using S^{35}-labelled proteins since it has been established that R.F. contains disulphide linkages disruption of which results in loss of activity (Franklin, Kunkel, et al, 1957).

SUMMARY

The British literature on Caplan's syndrome is briefly reviewed with particular reference to the pathogenesis of the pulmonary lesions and an investigation is described which was designed to throw fresh light upon this.

Statistical analysis of the results revealed an association between the levels of several serum protein fractions and the severity of the lung lesions independently of the severity of arthritis. Elevation of α2- and γ-globulins and reduction of albumin and of the albumin/α2-globulin ratio are consistent with the tuberculous theory of the aetiology of these lesions, but a trend was also observed towards higher levels of β-globulin with increasing severity of pulmonary lesions. Rheumatoid arthritis itself rarely produces this effect and no increase of β-globulin has been reported in tuberculosis.

The previously observed association between the presence of "Caplan lesions" in the lungs and positive haemagglutination tests for rheumatoid arthritis was confirmed and a significant correlation demonstrated between the agglutination titre and the β-globulin level.

The literature on the nature of the rheumatoid factor is reviewed and the conclusion reached that it exists naturally as a 19s macroglobulin migrating electrophoretically as a β2-globulin but that transformation to a 22s γ-globulin occurs when serum is stored in the liquid state.

Based on this conclusion and on the three-fold relationship observed between the severity of "Caplan lesions", the haemagglutination titre and the β-globulin level, a

hypothesis is proposed that the nodular pulmonary lesions described by Caplan and perhaps some other lesions associated with rheumatoid arthritis such as subcutaneous necrobiotic nodules may be sites of synthesis of the rheumatoid factor. It is further suggested that the raw material for this synthesis may consist of β-glycoprotein derived from the ground-substance of degenerating collagenous tissue.

Observations consistent with this hypothesis are quoted from the literature and several experiments are suggested which, if done, might support or refute the hypothesis.

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APPENDIX A

I have tried to determine whether there is a relationship between each of a number of serum protein percentages or one ratio of percentages and lung lesion grade in groups within which the arthritis grade is the same. In other words, I am trying to separate the variation with lung lesion grade from that with arthritis grade.

The regression within a group defined by an arthritis grade is called a "within-group regression", the independent variable being the lung lesion grade. A weighted average of these within-group-regressions is formed, and I have tested the significance of this average within-group regression coefficient. Values of regression coefficients and significance levels are summarised in the accompanying table.

There are some corollaries of the analysis that may be of interest to you. I have tested whether the individual within-group regression lines vary significantly. If the lines were the same, there would be no evidence for believing that arthritis grade affected the particular serum protein or ratio. Two types of difference in the lines have been tested statistically:-

(a) differences in regression coefficients (i.e. slopes)

(b) differences in positions of the lines.

The differences in slope are not significant, even at the 10% level, and we can test the significance of differences in position of the lines by considering the group means. First, if the lines are the same, the slope of the group means (regression of mean protein percentage against mean lung lesion) should be the same as the average within-group slope. Secondly, the

group means themselves should not deviate significantly from the regression line of the means. These two differences are tested and the significance levels found summarised in the last two rows of the table.

The analysis and interpretation of the data may be simplified by a consideration of the graph. I have taken the ratio A/α2 as an example. The regression lines are drawn over the range of observations (or of means) used to determine them. If there were no differences in the ratio A/α2 between arthritis grades, other than the variation with lung lesion grade, the within-group regression lines would be identical within themselves, and with the regression line of the group-means. As can be seen, the regression line of the means is steeper than the average within-group regression; it turns out that the slopes are significantly different at the 1% level.

In this example, the difference between regression lines within groups is due to group 0, for if the data are re-analysed, omitting group 0, neither the difference between the average within-group slope and the slope of means, nor the deviations of the group means from the regression of means is significant.

Three final remarks. Some of the tests are not very discriminating, and absence of significance does not imply that, with a larger sample, a difference would not be found. The analyses of different serum fractions are not independent because they are from the same men; this may affect your interpretation of the results. The regression coefficient of the means is always the same sign and greater than the average within-group regression. This is consistent with an

effect of arthritis in the same direction as that of lung lesions.

B. T. Warner.

23rd. June, 1960.

Summary of Analyses of Protein Fractions and One Ratio.

Dependent Variable		Albumin	α1-globulin	α2-globulin	β-globulin	γ-globulin	Albumin/ α2-globulin
Value of regression coefficient	Average within-group	- 4·1	0·5	1·1	0·8	2·4	- 1·4
	Regression of Means	-13·6	0·9	3·0	1·5	6·7	- 3·6
Significance Level of	Average within-group regression	·01<P<·025	·05<P<·10	·005<P<·01	·10<P<·25	·025<P<·05	·001<P<·005
	Between within-group regression coefficients	P>·25	P>·25	·10<P<·25	P>·25	P>·25	·10<P<·25
	Slope of Means v average within-group slope	·005<P<·01	P>·25	·01<P<·025	P>·25	·05<P<·10	·005<P<·01
	About regression of group means	·05<P<·10	·10<P<·25	·05<P<·10	P>·25	·025<P<·05	P<·001

Page

Date _____ Source of Data _____

Graph _____