ANTIBODY AND LYMPHOCYTE RESPONSES TO CELL NUCLEI IN HUMAN LUNG DISEASES.

Thesis submitted for the degree of

Doctor of Philosophy

in the

Faculty of Medicine

by

PATRICIA L. HASLAM

B.Sc., (Birmingham)

Cardiothoracic Institute

University of London

1976

ABSTRACT

This study has defined some of the characteristic antibody and lymphocyte responses to nuclear antigens in the lung diseases pleuropulmonary SLE, cryptogenic fibrosing alveolitis, asbestosis, sandblasters silicosis and intrinsic asthma. The aim was to see whether distinctive differences could be demonstrated which would support the proposal that the responses are stimulated in different ways in the different lung diseases, or alternatively whether there are similarities which would suggest that they are stimulated by a similar mechanism in response to antigens released from damaged lung cells.

The methods used to study prevalence, titres and immunoglobulin classes of antinuclear antibodies (ANA), and to detect complement fixing ANA and deoxyribonucleoprotein antibodies, were modifications of the indirect immunofluorescent staining technique. In addition, Ouchterlony double diffusion was used to detect precipitins to 'double' and single stranded deoxyribonucleic acid (DNA), and a modified Farr assay to detect antibodies binding with double stranded DNA.

Distinctive differences in the antinuclear antibody characteristics in the different lung diseases were demonstrated. In pleuropulmonary SLE, ANA titres were generally high, sera frequently contained multiple immunoglobulin classes of ANA, and complement fixing ANA and precipitins probably in most instances to single-

stranded DNA were often present. Antibodies inducing LE cell formation, and deoxyribonucleoprotein antibodies were detected in the majority, and some had antibodies binding with double stranded DNA although the frequency was lower than usual for groups of patients with SLE.

In cryptogenic fibrosing alveolitis, ANA titres were generally lower than in pleuropulmonary SLE, but many sera had multiple immunoglobulin classes of ANA, and complement fixing ANA and precipitins more probably to single stranded DNA were also common. However, antibodies inducing LE cell formation, antibodies to deoxyribonucleoprotein, and antibodies binding with double stranded DNA were very rare.

In asbestosis and silicosis sera tended to contain single immunoglobulin classes of ANA. In asbestosis this was usually lone IgM ANA, and ANA titres were low, complement fixing ANA was notably absent and only a small number of sera contained precipitins to single stranded DNA. Of especial interest was evidence suggesting an association between ANA production and aging. In silicosis, and in the other lung diseases, ANA occurred similarly in patients of young and old age, and in silicosis this, together with the usual finding of lone IgG ANA, suggested that the mechanism of ANA production is different from that in asbestosis. Complement fixing ANA was detected in only three silicotic sera, but precipitins to single stranded DNA were common.

In intrinsic asthma some sera had mixtures and others lone immunoglobulin classes of ANA, but tests with the various pure antigens were nearly all negative, and none of the sera had complement fixing ANA.

The peripheral leucocyte migration test was used to look for in vitro evidence of lymphocyte sensitization to various nuclear antigens. Again there were distinct differences of response in the different lung diseases. In pleuropulmonary SLE lymphocyte sensitization was demonstrated using isolated rat liver cell nuclei, DNA and deoxyribonucleoprotein. In contrast, in cryptogenic fibrosing alveolitis and asbestosis lymphocyte sensitization was demonstrated only with DNA. A small number of ANA positive patients with intrinsic asthma did not show lymphocyte sensitization to any of these antigens. (Silicosis was not included in the lymphocyte studies).

The patients with lymphocyte sensitization to DNA or deoxyribonucleoprotein were not necessarily those with antibodies to the same antigens, nor was antibody production invariably accompanied by lymphocyte sensitization.

The main conclusion was that the observations support the proposal that immune responses to various nuclear antigens are stimulated in different ways in different lung diseases.

Some theoretical stimulatory routes are discussed, particularly the possible importance of virus in pleuropulmonary SLE,

and the possible interplay between irritant dust and damage to intra-alveolar macrophages in asbestosis and silicosis.

Also discussed is the suggestion that the variable properties of these different responses might allow some to participate in tissue damage, for example particularly complement fixing ANA's or sensitized lymphocytes, but others to remain no more than innocuous markers of disease.

C O N T E N T S

ABSTRACT 3.

LIST OF ABBREVIATIONS 7.

AIM OF THE THESIS 9.

CHAPTER 1 - INTRODUCTION 10.

I. GENERAL INTRODUCTION 11.

II. LITERATURE REVIEW OF IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN LUNG DISEASE 14.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN CONNECTIVE TISSUE DISORDERS OF THE LUNG 14.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN INDUSTRIAL LUNG DISEASES 28.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN ASTHMA 37.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN BRONCHIAL CARCINOMA 39.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN CHRONIC BRONCHITIS AND SMOKING 40.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN LUNG DISEASE CAUSED BY INFECTIVE AGENTS 42.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN DRUG-INDUCED LUNG DISORDERS 43.

SUMMARY 46.

III. MAIN CONCLUSIONS FROM THE LITERATURE REVIEW 48.

CHAPTER 2 - PATIENTS AND CONTROLS 50.

I. PATIENTS AND CONTROLS USED TO STUDY PREVALENCE OF IMMUNOFLUORESCENT ANA 51.

PATIENTS WITH LUNG DISEASE 51.

PATIENT AGE 58.

CONTROLS WITHOUT LUNG DISEASE 58.

II. PATIENTS AND CONTROLS USED TO DEFINE THE CHARACTERISTICS OF ANA 61.

PATIENTS WITH LUNG DISEASE 61.

CONTROLS WITHOUT LUNG DISEASE 62.

III. PATIENTS AND CONTROLS USED TO STUDY LYMPHOCYTES 63.

PATIENTS 63.

CONTROLS 65.

CHAPTER 3 - MATERIALS AND METHODS 66.

I. ANTISERA FOR IMMUNOFLUORESCENT STAINING TESTS 67.

COMMERCIAL SOURCES 67.

CONJUGATION OF ANTISERA WITH FLUORESCEIN ISOTHIOCYANATE (FITC) 67.

TISSUE POWDER ABSORPTION OF FITC-CONJUGATES 70.

ABSORPTION OF FITC-CONJUGATED HORSE ANTIHUMAN COMPLEMENT (C3 AND C4) 71.

CONFIRMATION OF SPECIFICITY AND STANDARDIZATION OF FITC-CONJUGATED ANTISERA 71.

II. NUCLEASE ENZYMES 73.

NUCLEASE S1 73.

RIBONUCLEASE 73.

DEOXYRIBONUCLEASE 73.

III. NUCLEAR ANTIGENS 75.

NUCLEAR ANTIGENS FOR LYMPHOCYTE STUDIES 80.

IV. METHODS TO STUDY ANTINUCLEAR ANTIBODIES 85.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR ANTINUCLEAR ANTIBODIES (ANA) 85.

FLUORESCENCE MICROSCOPY 86.

PHOTOMICROGRAPHY 88.

METHODS TO REDUCE INTER AND INTRA-OBSERVER VARIABILITY IN IMMUNOFLUORESCENT TEST READING 88.

CONTROLS TO CONFIRM IMMUNOLOGICAL SPECIFICITY OF IMMUNOFLUORESCENT STAINING 89.

TITRATION OF IMMUNOFLUORESCENT ANA 90.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR IMMUNOGLOBULIN CLASSES OF ANA 91.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR COMPLEMENT-FIXING ANA 91.

LE CELL TESTS 92.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR ANTIBODIES TO DEOXYRIBONUCLEOPROTEIN 94.

METHODS TO DETECT ANTIBODIES TO DEOXYRIBONUCLEIC ACID (DNA) 94.

METHODS TO DETECT ANTIBODIES TO RIBONUCLEATE ANTIGENS 105.

V. METHODS TO STUDY LYMPHOCYTES 108.

LEUCOCYTE MIGRATION TEST (LMT) 108.

TRYPAN BLUE VIABILITY TESTS 109.

CONSTRUCTION OF DOSE RESPONSE CURVES 110.

CONTROLS FOR LYMPHOKINE INDEPENDENT INHIBITION 110.

ANTINUCLEAR ANTIBODY TESTS FOR CORRELATIONS 112.

CHAPTER 4 - RESULTS AND CORRELATIONS 114.

I. RESULTS OF STUDIES ON PREVALENCE OF IMMUNOFLUORESCENT ANA 115.

PREVALENCE OF IMMUNOFLUORESCENT ANA IN NORMAL CONTROLS 115.

PREVALENCE OF IMMUNOFLUORESCENT ANA IN LUNG DISEASE IN COMPARISON WITH NORMAL CONTROLS 118.

SELECTION OF LUNG DISEASE GROUPS FOR FURTHER STUDY 126.

II. TITRES OF IMMUNOFLUORESCENT ANA (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN) 128.

NORMAL CONTROL STUDIES 128.

LUNG DISEASE STUDIES 128.

III. IMMUNOGLOBULIN CLASSES OF IMMUNOFLUORESCENT ANA 137.

NORMAL CONTROL STUDIES 137.

LUNG DISEASE STUDIES 137.

CORRELATION BETWEEN THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA AND THE ANA TITRE (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN) IN ANA POSITIVE SERA 153.

IV. PATTERNS OF ANA IMMUNOFLUORESCENCE 157.

NORMAL CONTROL STUDIES 157.

LUNG DISEASE STUDIES 164.

CORRELATION BETWEEN PATTERNS OF ANA IMMUNOFLUORESCENCE AND THE ANA TITRE (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN) IN ANA POSITIVE SERA 169.

CORRELATION BETWEEN PATTERNS OF ANA IMMUNOFLUORESCENCE AND IMMUNOGLOBULIN CLASSES OF ANA 172.

V. COMPLEMENT FIXING ABILITY OF ANA 177.

NORMAL CONTROL STUDIES 177.

LUNG DISEASE STUDIES 177.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND ANA TITRES IN ANA POSITIVE SERA 186.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA 188.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA 190.

VI. ANTIBODIES REACTIVE WITH CALF THYMUS DEOXYRIBONUCLEOPROTEIN 194.

NORMAL CONTROL STUDIES 194.

LUNG DISEASE STUDIES 194.

CORRELATION BETWEEN DEOXYRIBONUCLEOPROTEIN ANTIBODIES AND LE CELL FORMATION 201.

CORRELATION BETWEEN REACTIVITY TO DEOXY-RIBONUCLEOPROTEIN AND THE ANA TITRES IN ANA POSITIVE SERA 202.

CORRELATION BETWEEN REACTIVITY TO DEOXY-RIBONUCLEOPROTEIN AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA 204.

CORRELATION BETWEEN REACTIVITY TO DEOXY-RIBONUCLEOPROTEIN AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA 205.

CORRELATION BETWEEN REACTIVITY TO DEOXY-RIBONUCLEOPROTEIN AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA 207.

VII. ANTIBODIES PRECIPITATING WITH CALF THYMUS 'DOUBLE STRANDED' DEOXYRIBONUCLEIC ACID ('DS'-DNA) 211.

RESULT OF AN ATTEMPT TO REDUCE SINGLE STRANDED (SS) DNA CONTAMINATION FROM COMMERCIAL CALF THYMUS 'DS'-DNA BY TREATMENT WITH NUCLEASE SI 211.

NORMAL CONTROL STUDIES 215.

LUNG DISEASE STUDIES 215.

CORRELATION BETWEEN OUCHTERLONY PRECIP-ITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE ANA TITRES IN ANA POSITIVE SERA 220.

CORRELATION BETWEEN OUCHTERLONY PRECIP-ITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE PROFILE OF IMMUNO-GLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA 220.

CORRELATION BETWEEN OUCHTERLONY PRECIP-ITATION TO NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE PATTERNS OF ANA IMMUNO-FLUORESCENCE IN ANA POSITIVE SERA 222.

CORRELATION BETWEEN OUCHTERLONY PRECIP-ITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA 225.

CORRELATION BETWEEN OUCHTERLONY PRECIP-ITATION TO NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND REACTIVITY TO CALF THYMUS DEOXYRIBONUCLEOPROTEIN IN ANA POSITIVE SERA 227.

VIII. ANTIBODIES PRECIPITATING WITH CALF THYMUS SINGLE STRANDED DEOXYRIBONUCLEIC ACID (SS-DNA) 230.

NORMAL CONTROL STUDIES 230.

LUNG DISEASE STUDIES 230.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE ANA TITRES IN ANA POSITIVE SERA 237.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA 239.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA 241.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA 241.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND REACTIVITY TO CALF THYMUS DEOXYRIBONUCLEOPROTEIN IN ANA POSITIVE SERA 243.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA IN ANA POSITIVE SERA 245.

IX. ANTIBODIES BINDING WITH 'DOUBLE-STRANDED' I^{125}-LABELLED HELA CELL DEOXYRIBONUCLEIC ACID ('DS' HELA DNA) 249.

NORMAL CONTROL STUDIES 249.

LUNG DISEASE STUDIES 249.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND ANA TITRES IN ANA POSITIVE SERA 253.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA 255.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA 257.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA 259.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND REACTIVITY TO DEOXYRIBONUCLEOPROTEIN 259.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA IN ANA POSITIVE SERA 262.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA IN ANA POSITIVE SERA 262.

X. RESULTS OF STUDIES TO DETERMINE WHETHER SPECKLED AND NUCLEOLAR IMMUNOFLUORESCENT STAINING PATTERNS ARE ASSOCIATED WITH ANTIBODIES TO RIBONUCLEATE ANTIGENS 266.

IMMUNOPRECIPITATION TESTS WITH DEPOLYMERIZED TOTAL YEAST RIBONUCLEIC ACID (RNA) 266.

CHANGES IN PATTERNS OF ANA IMMUNOFLUORESCENCE ON RIBONUCLEASE AND DEOXYRIBONUCLEASE PRE-TREATED RAT LIVER SECTIONS 268.

SUMMARY OF ANTINUCLEAR ANTIBODY RESULTS 271.

XI. RESULTS OF LYMPHOCYTE STUDIES 273.

INHIBITION OF LEUCOCYTE MIGRATION WITH NUCLEI, DEOXYRIBONUCLEOPROTEIN AND DNA 273.

ANTIGEN CONCENTRATION 282.

ENHANCEMENT OF LEUCOCYTE MIGRATION WITH NUCLEI, DEOXYRIBONUCLEOPROTEIN AND DNA 284.

CORRELATION BETWEEN LMI USING CRUDE NUCLEI, AND POSITIVE INHIBITION USING DEOXYRIBONUCLEOPROTEIN AND DNA 285.

SINGLE-STRANDED DNA AND DEPOLYMERIZED RNA 285.

CORRELATION BETWEEN ANA TITRE AND INHIBITION OF LEUCOCYTE MIGRATION 287.

CORRELATION BETWEEN LMT AND ANTIBODY RESULTS USING DNA AND DEOXYRIBONUCLEOPROTEIN 287.

EFFECT OF CORTICOSTEROID TREATMENT 290.

SUMMARY OF LYMPHOCYTE RESULTS 291.

CHAPTER 5 - DISCUSSION 293.

GENERAL DISCUSSION 294.

SENSITIZATION TO NUCLEAR ANTIGENS IN NORMAL CONTROLS 295.

SENSITIZATION TO NUCLEAR ANTIGENS IN 'PLEUROPULMONARY' SLE 300.

SENSITIZATION TO NUCLEAR ANTIGENS IN CRYPTOGENIC FIBROSING ALVEOLITIS 320.

SENSITIZATION TO NUCLEAR ANTIGENS IN ASBESTOSIS 331.

SENSITIZATION TO NUCLEAR ANTIGENS IN SILICOSIS 336.

SENSITIZATION TO NUCLEAR ANTIGENS IN INTRINSIC ASTHMA 339.

THE INFLUENCE OF CORTICOSTEROID THERAPY 340.

SUMMARIZED CONCLUSION 343.

BIBLIOGRAPHY 347.

ACKNOWLEDGEMENTS 378.

APPENDIX STATISTICAL METHODS AND TABULATED RESULTS 379.

TABLES

1	47.
2	59.
3	64.
4	119.
5	122.
6	123.
7	131.
8	135.
9	139.
10	142.
11	145.
12	154.
13	155.
14	171.
15	183.
16	185.
17	187.
18	191.
19	193.
20	198.
21	200.
22	203.
23	206.
24	208.
25	209.
26	212.
27	217.
28	219.
29	221.
30	223.
31	224.
32	226.
33	228.
34	234.
35	236.
36	238.
37	240.
38	242.
39	244.
40	246.
41	248.
42	251.
43	252.
44	254.
45	256.
46	258.
47	260.
48	261.
49	263.
50	264.
51	267.
52	269.
53	272.
54	274.
55	281.
56	286.
57	289.
58	292.

FIGURES

1	111.
2	116.
3	125.
4	129.
5	133.
6	136.
7	146.
8	150.
9	165.
10	167.
11	170.
12	173.
13	175.
14	189.
15	278.
16	279.
17	280.
18	283.
19	288.

PHOTOGRAPHIC PLATES

1	83.
2	99.
3	99.
4	101.
5	101.
6	103.
7	159.
8	159.
9	161.
10	161.
11	163.
12	179.
13	179.
14	181.
15	196.
16	196.
17	214.
18	214.
19	232.
20	232.
21	277.
22	277.

LIST OF ABBREVIATIONS

Ab	Antibody
AICF	Autoimmune complement fixing antibodies
ANA	Antinuclear antibodies
ASB	Asbestosis
AST	Intrinsic asthma
BTTA	British Thoracic & Tuberculosis Association
C	Control
CFA	Cryptogenic fibrosing alveolitis
Coal Pn.	Coal miners pneumoconiosis
DNA	Deoxyribonucleic acid
DNAase	Deoxyribonuclease
DS	Double-stranded
EAA	Extrinsic allergic alveolitis
EBM	Eagle's basal medium
EBMS	Eagle's basal medium + 20% foetal calf serum
F	Female
FITC	Fluorescein isothiocyanate
H	Homogeneous
If	Immunofluorescent
Ig	Immunoglobulin
LE cell	Lupus erythematosus cell
LMT	Leucocyte migration test
Log.	Logarithm

M	Male
MRC	Medical Research Council
N	Nucleolar
Neg (or -)	Negative
No.	Number
NS	Not significant
Nucleoprotein	Deoxyribonucleoprotein
p	Probability level
P	Peripheral
Pos. (or +)	Positive
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RNA	Ribonucleic acid
RNAase	Ribonuclease
SD	Standard deviation
SEM	Standard error of the mean
Sig. diff.	Significant difference
SIL	Silicosis
SJ	Sjo gren's syndrome
SLE	Systemic lupus erythematosus
Sp	Speckled
SS	Single-stranded
Stat.	Statistical

AIM OF THE THESIS

The object of this study was to confirm an increased prevalence of immunofluorescent antinuclear antibodies in patients with certain lung diseases by comparison with normal controls of similar age and sex. Following this to define some of the characteristic responses to nuclear antigens in such patients, including an investigation of cellular as well as humoral responses. Finally, to use the information obtained to discuss the proposal that immune responses to nuclear antigens arise in a number of different ways in lung diseases according to the distinctive clinical circumstances and are not a common consequence of any kind of extensive tissue destruction in the lung.

If distinct differences of response are confirmed in the different lung diseases it would then be possible to propose that their variable properties may allow some to play a role in tissue damage but others to remain no more than harmless immunological markers.

CHAPTER 1

INTRODUCTION

I. GENERAL INTRODUCTION

Immune responses to antigens of cell nuclei do not normally arise in humans or other animal species because of the existence of a natural state of immune tolerance to self-antigens (Dresser and Mitchison, 1968; Weigle, 1973; Allison, 1973; Howard and Mitchison, 1975). Occasionally however, they do occur, possibly as a result of defective immune tolerance to nuclear antigens, or to chance breakdown or bypass of tolerance. Their appearance must therefore be considered an abnormal phenomenon.

The practical need for research into such abnormalities in clinical medicine is that while they are only rarely present in disease-free individuals (Serafini, Torrigiani and Masala, 1964; Cannat and Seligmann, 1965; Whittingham et al, 1969), they are common in patients with certain diseases (Koffler et al, 1971; Tan, 1973). This implies that they might play a role in pathogenesis. Indeed, there is supportive evidence that antibodies reacting with nuclear antigens (ANA's) play a role in pathogenesis under some circumstances but not others. Most of this evidence has come from studies of human (Cochrane and Koffler, 1973; Nakamura and Allen, 1973; Koffler, 1974) and murine (Dixon, Oldstone and Tonietti, 1971; Howie, 1973; Talal and Steinberg, 1974) systemic lupus erythematosus (SLE)..

Circulating ANA's have been found in some patients with a number of different lung diseases, notably 'pleuropulmonary' SLE (Holgate et al,

1976), cryptogenic fibrosing alveolitis (CFA) (Turner-Warwick and Doniach, 1965), asbestosis (Turner-Warwick and Parkes, 1970) and skin test negative asthma (Hall, Turner-Warwick and Doniach, 1966). This suggests that lung damage in these patients might be exaggerated by immune hypersensitivity reactions involving nuclear antigens. In the main, this suggestion has not been explored, nor have detailed studies of the ANA's in lung disease previously been undertaken. However, there is one report presenting evidence of immune complex deposition in pulmonary capillaries in a minority of cases of CFA correlating with the presence of circulating ANA (Turner-Warwick, Haslam and Weeks, 1971). For these reasons, the present study has attempted to identify at least some of the types of ANA's which occur in lung disease and to determine whether any are of a type which potentially could take part in tissue damaging reactions in the lung.

Extensive lymphocytic infiltration is a feature of the histopathology of many of these lung diseases, and since there is evidence of lymphocyte sensitization to deoxyribonucleic acid (DNA) in patients with SLE (Azoury, Jones and Gum, 1966; Patrucco, Rothfield and Hirschhorn, 1967; Muller-Berat and Moulias, 1970), this study has also looked for evidence of lymphocyte responses to nuclear antigens in patients with lung disease. Should lymphocytes sensitized to nuclear antigens be detected, it would then be possible to propose that they also may initiate immune hypersensitivity reactions in the lung.

The ways in which immune responses to nuclear antigens are stimulated are in general unknown and it was hoped that the information provided by this study would support the proposal that in lung diseases they arise in a number of different ways dependent upon the distinctive clinical circumstances, and that in consequence they may contribute to the tissue damage in some, but not in other, lung diseases.

II. LITERATURE REVIEW OF IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN LUNG DISEASE

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN CONNECTIVE TISSUE DISORDERS OF THE LUNG

The idea that there might be a connection between certain types of damage in the lung and autosensitization to nuclear or other tissue antigens arose after it was recognised that the lung is frequently involved in connective tissue disorders of unknown etiology which are associated with the production of nuclear and other types of non-organ specific autoantibodies (Forbes, 1958; Mackay and Burnet, 1963; Scadding, 1967). Lung involvement within the connective tissue disorders has recently been reviewed by Turner-Warwick (1974) and will not be described in detail here. In brief, lung involvement can occur in systemic lupus erythematosus (Huang and Lyons, 1966), rheumatoid arthritis (Ellman and Ball, 1948), scleroderma (Weaver, Divertie and Titus, 1967), Sjogren's syndrome (Karlish, 1969), chronic active hepatitis (Turner-Warwick, 1968), dermatomyositis (Hyun, Diggs and Toone, 1962) and polymyositis (Weaver et al, 1968). Not only do these disorders have similar immunological features, but there is often clinical overlap which suggests a common etiologic agent, or some other common feature, which might also relate to the lung changes.

Lung Disorders Associated with Systemic Lupus Erythematosus (SLE)

The most common type of lung involvement in SLE is bilateral pleural effusion and pleural inflammation (Harvey et al, 1954). In addition, linear shadows over the lower zones are a common radiographic appearance, some of which may reflect pulmonary vasculitis (Hoffbrand, 1965).

Alveolar wall fibrosis in SLE is very uncommon but has occasionally been seen (Israel, 1953; Olsen and Lever, 1970).

The first demonstration of an immune reaction involving nuclear antigens in human disease was the discovery of LE cells in bone marrow cell smears from patients with SLE (Hargraves, Richmond and Morton, 1948). Lung abnormalities were described amongst other clinical abnormalities in these patients and this is, therefore, also the first report of an immune response involving nuclear antigens in patients with lung disorder.

The ANA's in patients with SLE have been studied in considerable detail (Alarcón-Segovia et al, 1970; Reichlin and Mattioli, 1974). Particularly important are studies which have implied that under certain circumstances ANA's play a role in pathogenesis. Immunoglobulins and complement have been demonstrated within the glomerular capillary bed of kidneys from patients with SLE nephritis (Freedman and Markowitz, 1962; Paronetto and Koffler, 1965), and occasionally fixed to renal cell nuclei (Hench, Tan and Wilson, 1969). Moreover, antinuclear antibodies have been eluted from such sites (Krishman and Kaplan, 1966; Krishman and Kaplan, 1967; Koffler, Schur and Kunkel, 1967; Koffler, Agnello and Kunkel, 1974). Immunoglobulin and complement have also been demonstrated at the dermal-epidermal junction (Vasquez and Dixon, 1957; Cormane, 1964; Grossman, Callerame and Condemi, 1974; Gilliam et al, 1974) and occasionally localised to the nuclei of

epidermal cells (Tan and Kunkel, 1966) in biopsies from skin lesions of patients with SLE. Circulating antibodies to double-stranded DNA have been reported varying with serum complement levels and reflecting clinical activity of the renal disease (Tan et al, 1966; Schur and Sandson, 1968; Webb, Whaley and Lee, 1974). This evidence led to proposals that Type III hypersensitivity reactions, initiated by local immune-complex formation or circulating immune-complex deposition within capillaries with subsequent triggering of the complement cascade (Coombs and Gell, 1975), may contribute to the lesions at these sites.

It is not known whether immune hypersensitivity reactions involving nuclear antigens contribute to lung damage in SLE, but Turner-Warwick (1974) reported two observations which support this possibility. In one patient with SLE having intra-alveolar wall fibrosis, immunofluorescent studies showed immunoglobulin and complement within alveolar capillaries suggesting that at least in this patient the lung damage might be the consequence of immune complex deposition. In another patient, immunoglobulin and complement were observed on the nuclei, or within the cytoplasm, of mesothelial cells obtained from the pleural fluid. The evidence in this case suggests that ANA may aggravate pleural damage. Hughes and Rowell (1970) showed that a turpentine induced pleurisy in rats could be aggravated by intra-pleural infusion of human serum containing high titre ANA. Immunoglobulin and complement were demonstrated on the nuclei or in the cytoplasm of mesothelial cells in the pleural fluid of these rats, and it was suggested that ANA forms immune complexes with the nuclei of dead cells which when taken up by phagocytic cells initiate further inflammatory changes.

Recently Holgate et al (1976) have examined the clinical features of the rather special group of patients with SLE collected for inclusion in this study who presented at the Brompton Hospital with pleuropulmonary or cardiac symptoms. The incidence of most clinical symptoms was similar to that reported for other groups of SLE, but there was an unusually low incidence of renal involvement. Immunologically, all the patients had LE cells and immunofluorescent ANA, and precipitating antibodies reacting with commercial calf-thymus DNA were present in the majority. There was, however, an unusually low incidence of positive binding with I^{125} HeLa cell DNA in a Farr-assay type of technique which is normally positive in the majority of patients with SLE. (Holian et al, 1975). It was suggested that the immunological finding of a low incidence of 'double-stranded' ('DS') DNA binding might reflect the clinical finding of a low incidence of renal involvement.

In vitro studies using tests for which there is at least some correlation with cell-mediated immunity have shown evidence of lymphocyte sensitization to DNA in patients with SLE. Inhibition of the migration of peripheral blood leucocytes from patients with SLE in the presence of DNA has been demonstrated (Muller-Berat and Moulias, 1970; Moulias et al, 1970; Harpey et al, 1971; Galanaud et al, 1971), and also blast transformation of lymphocytes from patients with SLE in the presence of DNA (Patrucco, Rothfield and Hirschhorn, 1967; Reinert et al, 1971). Highly polymerised calf thymus DNA was used in all of these studies, and in addition highly polymerised fish DNA was used by Moulias et al, (1970) and Reinert at al, (1971). Evidence of delayed hypersensitivity after intracutaneous skin tests with homologous DNA, deoxyribonucleoprotein and histone (Azoury, Jones and Gum, 1966) and with highly polymerised calf thymus DNA (Reinert et al, 1971) has also been obtained in patients with SLE.

The role, if any, of lymphocytes sensitized to nuclear antigens in disease pathogenesis is, however, not known, nor are methods yet

available to determine whether lymphocytes with such sensitization are present in the lungs and able to stimulate cell-mediated hypersensitivity reactions which contribute to the lung damage.

Lung Disorders Associated with Rheumatoid Arthritis (RA)

The main types of lung involvement in rheumatoid arthritis are pleural effusion, necrobiotic intrapulmonary nodules and fibrosing alveolitis (Scadding, 1969; Turner-Warwick, 1969). But, although circulating rheumatoid factor (RF) is the classic immunological abnormality antinuclear factors (ANA's) occur in about 19% of patients (Ward, Johnson and Holborow, 1964; Glynn, 1975).

Evidence of immune complexes around the joints and in the joint fluids of patients with RA (Winchester, Agnello and Kunkel, 1970) has suggested a role for immune complexes in the pathogenesis of the joint lesions. Whether such complexes involve RF in some patients, or ANA in others, is not known. The demonstration that DNA may be released into the synovial fluid as a consequence of joint inflammation of various kinds (Hughes et al, 1971) suggests that free antigen might be available to allow immune-complex formation in the joints of patients with circulating ANA.

Studies to investigate the possibility that autoantibodies may contribute to lung damage in RA are few. Turner-Warwick (1967) found immunofluorescent evidence of IgM and RF (using a conjugate of heat-

aggregated IgG) in the alveolar walls of a patient with fibrosing alveolitis in association with RA. It was suggested that this might indicate the deposition of immune complexes involving RF in this lung. The patient also had circulating high titre RF but tests for ANA were negative. More recently DeHoratius, Abruzzo and Williams (1972) have observed similar findings in five other patients.

Cryptogenic Fibrosing Alveolitis (CFA)

Fibrosing alveolitis (wide-spread inflammatory cell infiltration and alveolar wall fibrosis) (Scadding, 1964) is the most usual histopathological abnormality in the lungs in connective tissue disorders other than SLE and RA (Turner-Warwick, 1973).

Peabody et al (1950) were the first to suggest that CFA might be of allergic etiology on the basis of the demonstration of eosinophils in the interstitial tissues. The presence of eosinophils within the alveoli was also put forward as evidence compatible with the idea of an allergic pathogenesis for CFA by Read (1958). The first anti-tissue antibody reported in CFA was RF (Franklin, Zucke-Franklin and McEwen, 1959), and the first indication that ANA's might be present was the detection of LE cells in one case by Braunsteiner, Egghart and Potuzhek, 1960).

Cryptogenic fibrosing alveolitis occurs as a 'lone' disease as well as a part of a systemic connective tissue disorder, and it was there-

fore possible to examine the idea that the lung syndrome itself might be related etiologically to the other connective tissue disorders. Turner-Warwick and Doniach (1965) looked for evidence of immunological abnormalities in CFA similar to those which can be demonstrated in other connective tissue disorders. They studied a group of 48 patients with CFA and demonstrated an increased prevalence of ANA's (28%), RF's (49%) and non-organ specific autoimmune complement-fixing autoantibodies (AICF) (19%). The antibodies occurred in patients with the 'lone' disease and in those with systemic involvement providing immunological evidence favouring a relationship with other connective tissue disorders. The ANA's were detected by immunofluorescence and were more common in women. Only six of the fourteen patients with ANA had evidence of other types of autoimmune systemic disease. LE cells were not demonstrable in any patient, and ANA titres tended to be lower than in SLE. The pattern of nuclear immunofluorescence was diffuse in 12 and speckled in 2, one also with nucleolar staining. The 26 sera with negative immunofluorescent ANA were also negative in a test for leucocyte specific ANA which has been found in occasional cases of RA and Felty's syndrome (Faber et al, 1964).

Mackay and Ritchie (1965) also looked for autoimmune abnormalities in CFA stimulated by the evidence presented by Read (1958) that the Hamman-Rich syndrome, as it was then called, is based on immune mechanisms. They purposefully excluded patients with associated systemic disease and in 17 patients studied found immunofluorescent

ANA's in 41% (7/17), RF's in 24% (4/17), AICF in 18% (3/17) and antithyroglobulin antibodies in 18% (3/17). ANA titres were not reported but the immunofluorescent staining was described as 'weak' in distinction from the strongly positive staining usually reported in SLE.

In an extended series of 109 patients with CFA Turner-Warwick (1967) reported immunofluorescent ANA in 31% (34/109). Again, the ANA's appeared to differ from those in SLE. LE cell tests were negative in all but 2 patients, and ANA titres ranged from 1/10 to 1/400, but only a third had titres of over 1 in 50. In addition, most of the ANA's were destroyed by heating serum at 65^{o}C for 30 minutes indicating that they may usually be of the IgM immunoglobulin class rather than the heat stable IgG ANA more commonly found in SLE (Weir and Holborow, 1962). Furthermore, none of these patients had clinical evidence of SLE. RF was present in 31% (34/109) of the patients only 7 of whom also had ANA, and AICF in 19% (21/109) the majority of whom also had either ANA or RF.

Turner-Warwick (1967) also studied a lung from one patient with CFA who had an ANA titre of 1/200, and showed evidence of IgM and IgA within plasma cells, and complement as well as IgM and IgA within occasional intra-alveolar macrophages. This was the first evidence suggesting a role for immune complexes, possibly involving ANA, in

the pathogenesis of the disorder. It was suggested that such complexes may have been deposited in this lung and subsequently phagocytosed by intra-alveolar macrophages.

In another study, Hobbs and Turner-Warwick (1967) found that the presence of various non-organ specific antoantibodies in 23 patients with CFA was associated with selective increases in quantitative serum immunoglobulin levels in 19 patients. Serum immunoglobulin levels were normal in 6 of 9 patients without autoantibodies. It was not possible to relate individual autoantibodies to different immunoglobulin class increases because more than one type of autoantibody or immunoglobulin class increase could occur in a single serum.

Hobbs and Turner-Warwick (1967) also used monospecific antisera in the immunofluorescent test for ANA, and found that 6 of 12 patients with ANA had mainly IgG ANA and 6 others mainly IgM ANA. Four, however, had a suggestion of ANA in both immunoglobulin classes. This evidence of a multiplicity of ANA classes in single sera was suggested to reflect either a multiplicity of stimulating nuclear antigens as had been proposed previously in SLE by Barnett et al (1964), or possibly a persistent antigenic stimulation triggering more than one immunoglobulin class over a prolonged period of time.

Turner-Warwick (1968) described a group of 8 patients who had CFA in association with chronic liver disease, either chronic active

hepatitis or intralobular hepatitis. These liver disorders are also of unknown etiology and are associated with non-organ specific autoantibodies including ANA (Doniach et al, 1966; Walker et al, 1965). Eight patients were studied and 5 had serum ANA. Only one patient had LE cells.

In 1971 Turner-Warwick and Haslam considered the suggestion that autoantibodies may result from any type of chronic destructive fibrosing lung disease and presented evidence that this was an unlikely explanation. They did this by comparing autoantibody production in groups of patients with CFA and asbestosis with the situation in a group of patients with chronic extrinsic allergic alveolitis due to the inhalation of organic dusts (Pepys, 1967, 1969). All the patients showed features of advanced fibrosis. The increased prevalence of ANA and RF in the patients with CFA and asbestosis was reconfirmed, the prevalence of both types of antibody slightly lower in asbestosis than in CFA. The increased prevalence of AICF's was reconfirmed in CFA but it was discovered that the prevalence of AICF's was not increased in asbestosis. In chronic extrinsic allergic alveolitis, despite the extensive lung fibrosis, the prevalence of all these non-organ specific autoantibodies was very low and similar to that in random normal populations. No evidence of lung specific antibodies could be demonstrated in any disease. It was concluded that because of the differential distribution of various non-organ specific autoantibodies in the different fibrosing lung diseases their presence could not necessarily be explained as a non-specific consequence of all types of chronic destructive lung fibrosis.

The study of Turner-Warwick and Haslam (1971) also included a more detailed examination of the ANA's which were present in 53 (36.5%) of this extended group of 145 CFA patients. It was confirmed that ANA's are rather more common in females with CFA (45.4%) than males (31.5%). It was also confirmed that a diffuse pattern of nuclear immunofluorescence is the most common staining pattern. It was seen in 42 of the 53 ANA positive patients, a speckled pattern was seen in 7 and a membrane pattern in 2. One patient had a combined diffuse and speckled pattern and another had a combined membrane and speckled pattern. Twenty seven ANA positive sera were examined in the immunofluorescent test for ANA using monospecific antisera to human IgG and IgM. Eight had only IgG ANA, 7 only IgM ANA and 12 had a mixture of IgG and IgM ANA's. Thus it was confirmed with greater certainty that sera from patients with CFA frequently contain more than one immunoglobulin class of ANA. RF was present in 31.1% of the patients, and again it was seen that only rarely did ANA and RF occur together in the same serum.

Nagaya, Buckley and Sieker (1969) reported ANA in a large group of patients presenting with unexplained pulmonary fibrosis. They studied 20 of the ANA positive sera in more detail. None of the 20 had evidence of exposure to irritant dusts, or of associated systemic autoaggressive disease. The majority (18/20) had IgM ANA and 6 also had an equally high titre of IgG ANA. The remaining 2 reacted only with IgG. Just one of the 20 reacted with IgA as well as with IgG and IgM. There was no corr-

elation between the immunoglobulin classes of ANA and quantitative serum immunoglobulin levels which were not strikingly abnormal, contradicting the earlier finding of Hobbs and Turner-Warwick (1967).

Turner-Warwick (1974) made a clinical assessment of the large group of 151 patients with CFA who had been studied for non-organ specific autoantibodies at the Brompton Hospital. It was reconfirmed that autoantibodies occur in patients with and without associated systemic involvement. The incidence of ANA was as great in those with 'lone' CFA (28%) as in those with systemic involvement (24%). However, the incidence of RF in the lone disease was only slightly above that in the normal population (9.2% compared with an expected 5% approximately). There were 54 patients with associated systemic disease and RF was present in 23 (42.5%) of the 54 cases. Nineteen of the 23 had clinical evidence of transient polyarthritis or rheumatoid arthritis.

In 1971 Turner-Warwick, Haslam and Weeks reported immunofluorescent studies on lung biopsies from 33 cases of CFA. Immunoglobulin and complement immunofluorescence suggesting immune-complex deposition in the alveolar capillary walls was observed in 6 of 14 lungs from patients with circulating serum ANA, but in contrast the findings were negative in lungs from 19 patients without serum ANA. There was no association with any other group of circulating non-organ specific autoantibodies. Interestingly, one patient with CFA in association with SLE and with liver and kidney involvement, had evidence of immune-complex deposition within the kidney as well as the lungs. This observation suggested

that immune-complex deposition associated with ANA may account for alveolar wall damage in a small number of cases of fibrosing alveolitis, possibly by a mechanism similar to that suggested by Freedman and Markowitz (1959) for SLE nephritis.

In the same study, (Turner-Warwick, Haslam and Weeks; 1971) intra-alveolar macrophages containing immunoglobulin were demonstrated in 10 cases. Complement was also present within macrophages in 7 of the ten. Serum was available from 8, and 6 had circulating ANA. The immunoglobulin and complement was usually demonstrated in the cytoplasm of the intra-alveolar macrophages suggesting that immune complexes had been phagocytosed by these cells after deposition in the lungs. Indeed, macrophage fluorescence was seen in five of the six lungs where there was also evidence of immune complex deposition in the alveolar walls. Four lungs showed immunofluorescence associated with macrophage cell nuclei, and it was suggested that ANA might also be directly complexing with the nuclei of dead intra-alveolar macrophages possibly causing an acceleration of tissue damage.

Methods have not yet been applied to identify the type of antibody and antigen in immune-complex deposits in the lungs of patients with CFA similar to those which have been applied to elute and identify nuclear antibodies and antigens from kidney and skin lesions of patients with SLE.

More recent reports of ANA in CFA are those of Stack, Choo-Kang and Heard (1972) and Kang et

al (1973). Stack and his co-workers (1972) studied 62 patients with 'lone' CFA and found ANA in 30% and RF in only 13%. This is in keeping with Turner-Warwick's (1974) finding of ANA in 28.6% and RF in only 9.2% of patients with 'lone' CFA, contrasting with expected normal population levels of approximately 2% for ANA and 5% for RF. Stack's group also found that autoantibodies (either ANA or RF) occurred in equal frequency in young and older age groups, and were not associated with a better prognosis.

Summary: The main facts concerning ANA in CFA gleaned from the earlier literature can now be summarized.

1. There is an increased prevalence of immunofluorescent ANA in CFA which is similar in patients with 'lone' disease and in those with an associated systemic connective tissue disorder.

2. The increased prevalence of ANA is unlikely to be the result of any type of chronic fibrotic lung damage.

3. There is evidence that the ANA's in CFA differ from those in SLE generally having lower ANA titres and a higher frequency of IgM ANA.

4. Other groups of autoantibodies detected in CFA include RF's AICF's, thyroid and lung-reactive, but non-organ specific, auto-antibodies.

5. Immunofluorescent studies of lung tissues from patients with CFA have identified immunoglobulins and complement in a minority of cases, often associated with ANA in the serum.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN INDUSTRIAL LUNG DISEASES

Increases in the prevalence of ANA have also been reported in the fibrotic inorganic dust pneumoconioses asbestosis, coal miners' pneumoconiosis and silicosis.

Asbestosis

The first immunological abnormality reported in asbestosis was RF in between 13 and 21% of asbestos workers, and in up to 37.1% of those with abnormal chest radiographs (Pernis, Vigliani and Selikoff, 1965).

Turner-Warwick and Parkes (1970) obtained similar findings and also demonstrated an increased prevalence of ANA in 23 (28.8%) of a group of 80 asbestos workers which included 52 with abnormal chest radiographs. The ANA titres were lower than in SLE and similar to those in CFA; 13 (57%) of the 23 positive sera having titres of less than 1 in 40. Lone IgM ANA was present in 8 of the 23 positive sera, and mixed IgM and IgG ANA's in 14 but with an IgM predominance shown by titration. Only one serum had lone IgG ANA. The IgM ANA predominance suggested a resemblance with the IgM ANA's in connective tissue disorders such as RA, Sjogren's syndrome and systemic sclerosis (Holborow and Johnson, 1965).

Turner-Warwick and Parkes (1970) found no differences in age, sex or smoking habits in workers with circulating autoantibodies compared with

those without antibodies. ANA's were, however, found more frequently in those with clinical asbestosis compared with asbestos workers having normal lung radiographs. These observations were confirmed by Turner-Warwick (1973) and Soutar, Simon and Turner-Warwick (1974). The lack of relationship between tissue antibody production and duration of exposure to asbestos dust, but the correlation with radiographic abnormality suggested that the antibodies may play a secondary role in pathogenesis, or alternatively may be a consequence of tissue destruction. It was also suggested that some members of the population with a genetic predisposition to produce tissue antibodies may be selected out into the group with lung disease.

Turner-Warwick and Parkes (1970) conclusion that enhanced tissue antibody production in asbestosis is unlikely to be the consequence of prolonged dust irritation alone was supported by Turner-Warwick and Haslam (1971). They showed that ANA was present in 25% (19) and RF in 22.6% (17) of a group of 75 patients with extensive radiographic abnormality. However, in a control group of 75 asbestos workers matched with the patients for duration of exposure to asbestos dust as well as for age and sex but with normal lung radiographs, the prevalence of ANA and RF was very low (2.6% for both) and similar to expected random normal population levels.

Turner-Warwick and Haslam (1971) examined the ANA's in these patients with asbestosis in some detail and found that like those in CFA, they

usually show a diffuse pattern of nuclear immunofluorescence, although a speckled pattern was seen in two instances. The ANA titres were fairly low, 53% having titres of less than 1 in 40. The predominance of IgM ANA over IgG ANA was confirmed. Fourteen of 18 positive sera had only IgM ANA, two only IgG ANA and two others had a mixture of IgM and IgG ANA's. Although both ANA and RF occur in patients with asbestosis, as in CFA, they rarely were found together in the same serum.

In 1971 Turner-Warwick, Haslam and Weeks studied lungs from 8 patients with asbestosis for evidence of antibodies and complement using immunofluorescent methods. The results contrasted with those obtained in 33 lungs from patients with CFA in that there were no germinal follicles and plasma cells, nor evidence of immune-complex deposition in alveolar capillary walls. However, in 3 lungs there were occasional large intra-alveolar mononuclear cells containing immunoglobulin, and in 2 lungs also containing complement. The macrophage fluorescence was cytoplasmic, not nuclear, suggesting possible phagocytosis of immune-complexes. One of the 3 patients with macrophage immunofluorescence had circulating ANA of titre 1 in 80 and also RF of titre 1 in 512.

It is not yet clear whether progression is more rapid in patients with ANA than in those without, and preliminary reports on small numbers of cases have shown somewhat conflicting results (Turner-Warwick, 1973; Soutar, Simon and Turner-Warwick, 1974). Whether ANA is a

marker of disease, or an accelerating factor in the disease can only be answered completely by long term follow-up studies of asbestos exposed populations.

ANA occurs in about 5% of random normal populations and the prevalence increases with age (Cannat and Seligmann, 1965; Whittingham et al, 1969). Age alone, however, could not account for the increased prevalence of ANA in asbestosis, when compared with age-matched asbestos workers with normal chest radiographs where the prevalence was little different from that expected in the normal population. (Turner-Warwick, 1973).

Kang et al (1973) have recently confirmed the increased prevalence of ANA in asbestosis demonstrating immunofluorescent ANA in 11 (34.4%) of 33 patients. They also reported an increased prevalence of RF's and C-reactive protein. The most recent report is that of Lange et al (1974) who detected ANA's in 8 of 16 patients with asbestosis, but in only 4 of 20 workers with prolonged dust exposure but normal lung radiographs. This again suggests that ANA production is not a consequence of prolonged dust exposure alone.

Further study of the ANA's in asbestosis is particularly important in view of a possibility that they might play a secondary role stimulating accelerated tissue damage.

Coal Miners' Pneumoconiosis

Evidence that immunological host factors might influence the tissue changes induced by inhalation of coal dust was first described by Caplan (1953). He noted that 51 out of 14,000 claimants for disablement benefit were suffering from rheumatoid disease, and that 90% of these had complicated pneumoconiosis. The majority showed features of progressive massive fibrosis, but 25% showed a peculiar radiographic change which consisted of nodular well-defined opacities 0.5 to 5.0 cm. in diameter, distributed through both lung fields and now known as 'Caplan's nodules'. The histology of these nodules was similar to subcutaneous rheumatoid nodules (Gough, Rivers and Seal, 1955).

Caplan, Payne and Withey (1962) found a high incidence of serum RF not only in cases of coal pneumoconiosis with rheumatoid arthritis, but also in 70.6% of those with Caplan's nodules but without clinical arthritis. In addition, an increased prevalence of RF has been demonstrated when cases with progressive massive fibrosis are considered alone (Wagner and McCormick, 1967).

Soutar, Turner-Warwick and Parkes (1974) detected ANA in 17% of a group of 109 miners with coal pneumoconiosis and demonstrated a significant trend of increasing frequency of ANA with advancing radiographic severity. The prevalence was lowest in miners with simple pneumoconiosis 9% (3 out of 32) and increased slightly to 14% (4 out

of 28) in those with progressive massive fibrosis category A. Further increases were seen in category B, 24% (9 out of 38), and in category C, 27% (3 out of 11). RF was found in significant titre in 10% of the total 109 patients, and a trend to increase with increasing severity was also seen but less marked than for ANA.

The increased prevalence of ANA in coal miners has been confirmed by Lipmann et al (1973) who demonstrated ANA in 34% of 156 miners with coal pneumoconiosis; 9% had RF. In their studies the prevalence of ANA was highest in anthracite miners with complicated pneumoconiosis.

There have been three reports of direct attempts to find evidence of autoantibodies in the vicinity of 'Caplan nodules' and progressive massive fibrosis lesions. Wagner and McCormick (1967) studied biopsy and post mortem lungs from patients with coal pneumoconiosis using immunofluorescent methods. They demonstrated plasma cells containing immunoglobulin and some containing RF (using a conjugate of aggregated gamma globulin) in occasional lungs, both around Caplan nodules and within the massive fibrotic lesions. Pernis (1963) and Pernis et al (1965) have also demonstrated deposits of immunoglobulin, and in addition complement within Caplan nodules.

The literature concerning ANA production in complicated coal pneumoconiosis, although limited, is additional information emphasizing the need for further work to explain the nature of the relationship between

the presence of ANA and RF and the development of fibrosis in inorganic dust diseases.

Silicosis

The first worker to suggest that immunological phenomena might be involved in silicosis was Habeeb (1945) on the basis of the eosinophilia seen in this disease, and in 1959 Vigliani and Pernis also reviewed evidence in favour of this idea.

Falon (1937) demonstrated in experimental animals, and later Allison Harington and Birbeck (1966) in tissue culture models, that the initial fibrogenic response in silicosis appears due to macrophage cell death following phagocytosis of the highly toxic silica particles. To explain the conglomerate lesions which frequently develop in silicosis Vigliani and Pernis (1959) put forward the theory that these are the result of the superimposition of additional fibrogenic stimulating factors, possibly hypersensitivity reactions.

Vigliani and Pernis (1958) outlined an 'antigenic' theory for silicosis postulating that quartz combines with serum proteins to form a sensitizing antigen which is retained for a long period of time and against which antibody is slowly produced. The persistence of the silica-protein antigen would explain the persistence and progression of silicosis after removal of the patient from dust contact. They also suggested that the extensive lesions which develop when tuberculosis occurs in silicosis are due to the adjuvant effect of the tubercle bacilli enhancing the immunological response to the silica-protein antigen.

Evidence that antitissue responses might indeed contribute to the pathogenetic process in silicosis has been provided by reports of autoantibodies in some silicotic sera. The common occurrence of lung-reactive antibodies in patients with silicosis in contrast with their extreme rarity in unexposed controls has been demonstrated by Kalman, Mandi and Osztovics (1962) and Dinu and Roth (1965). In addition an increased prevalence of lung-reactive antibodies has been described in miners exposed to silica dust but without evidence of disease (Barbu et al, 1969).

Burrell and his co-workers have subsequently investigated lung-reactive antibodies in silicosis in considerable detail and concluded that they are non-organ specific and that collagen may be an important antigen in many instances (Burrell, Wallace and Andrews, 1964; Hagadorn and Burrell, 1968).

An increased prevalence of RF has been demonstrated in the serum of patients with silicosis by Gambini (1959) Pernis, Vigliani and Gambini (1963) and more recently by Kamijima (1968) and Kang et al (1973). Kamijima (1968) reported that RF reflected associated rheumatoid arthritis in some patients, and this clinical association has also been described by Spinazzola et al (1968). There have also been occasional reports of patients with silicosis developing other connective tissue disorders which are associated with non-organ specific autoantibody production (Ambrosi, 1966).

The first report suggesting the presence of ANA in silicotic sera was that of Kamijima (1968) who described the clinical follow-up of a group of patients. LE cells were detected in 5 and two developed evidence of autoallergic disorder during the course of their silicosis, one SLE and the other polymyositis. It was suggested that silica dust might act as an adjuvent in autoimmunization.

Kang et al (1973) have described immunofluorescent ANA in 25.8% (8 out of 31) patients with silicosis, and Jones et al (1976) have recently shown ANA in 14 (36%) of a group of 39 patients with sand-blaster's silicosis, the majority showing strongly positive nuclear immunofluorescent staining. A preliminary clinical analysis of this last group has shown that, as in asbestosis and coal pneumoconiosis the prevalence of ANA in silicosis increases with increasing radiographic severity. The question whether accelerating disease promotes ANA or whether ANA acts as an accelerator in inorganic dust pneumoconiosis remains unresolved.

Conclusion: In conclusion, since the literature suggests that the prevalence of ANA increases in patients with inorganic dust pneumoconiosis in relation to radiographic severity several important questions are posed:

1. Is there a genetic predisposition to produce tissue antibodies which may be prematurely expressed under the influence of dust irritation?

2. Is the mechanism of ANA production in these diseases similar to that causing ANA in disease-free ageing individuals?
3. Alternatively, is ANA produced by distinct mechanisms relating to the distinct properties and biological effects of each type of inhaled dust?
4. Whatever the mode of production, is the ANA innocuous or does it take part in immune hypersensitivity reactions which can aggravate fibrotic tissue damage?

To gain answers to these questions, more information must be obtained about the characteristics and properties of ANA's in the different industrial lung diseases.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN ASTHMA

Skin test negative asthma (intrinsic asthma) is occasionally associated with thyroid disease, rheumatoid disease and pernicious anaemia. (Hall, Turner-Warwick and Doniach, 1966; Jayson et al, 1967). There is also evidence of an increased prevalence of auto-antibodies including ANA's in intrinsic asthma which has recently been reviewed by Turner-Warwick (1974b).

Hall, Turner-Warwick and Doniach (1966) demonstrated an increased prevalence of thyroid, gastric and ANA antibodies in females with

intrinsic asthma, but a low prevalence in age and sex matched patients with extrinsic asthma. The prevalence of ANA was 21% (7 out of 38) in females with intrinsic asthma, compared with 0 out of 32 age matched females with extrinsic asthma and 0 out of 38 aged matched normal healthy females. In our own series of patients ANA occurred in 16% of 58 patients with intrinsic asthma compared with 0% of 49 patients with extrinsic asthma (Turner-Warwick and Haslam, 1970; Turner-Warwick, 1974b). Chachaj et al (1973) described ANA in 20% of 30 patients with severe intrinsic asthma compared with only 4% in a group of healthy controls, but they did not study patients with extrinsic asthma.

Other workers, however, have been unable to demonstrate any increase in the prevalence of ANA in patients with asthma, either in those with negative or those with positive skin reactions (Serafini, Torrigiani and Masala, 1965; B.T.T.A., 1974).

Turner-Warwick (1976) put forward the suggestion that the slightly enhanced production of autoantibodies reported by some workers in intrinsic asthma might reflect some type of viral infection for which there is some suggestive clinical evidence. This might cause impaired lymphocyte surveillance which might in turn lead to autoantibody production in older patients, and in younger patients with a genetic predisposition to develop autoantibodies.

In conclusion, although the literature does not indicate an important

role for ANA in intrinsic asthma, intrinsic asthmatic sera were included in the present study for the interest of examining the variety of routes by which ANA might arise in different lung diseases.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN BRONCHIAL CARCINOMA

Following the report of Whitehouse and Holborow (1971) of an increased prevalence of ANA (24%) as well as smooth muscle antibody (67.5%) in 80 patients with malignant diseases Hodson and Turner-Warwick (1975) looked for evidence of autoantibodies in patients with bronchial carcinoma. They observed no overall increase in the prevalence of ANA, smooth muscle or reticulin autoantibodies in a group of 105 patients with bronchial carcinoma of various histological types, but found a highly significant increase in the prevalence of ANA in the patients with adenocarcinoma (31%) compared with age and sex matched controls (5%). There was no association with age, sex, smoking or chronic bronchitis. Several explanations were suggested for the increased prevalence of ANA in adenocarcinoma. Adenocarcinoma of the bronchus is not related to smoking and ANA production might relate to persistent oncogenic virus. Alternatively, autoantibody production might be the result of a general T-lymphocyte deficiency which has been noted in many types of malignant diseases and which might lead to inefficient lymphocyte surveillance. It was also suggested that stimulation of lymphocytes by tissue antigens might facilitate the replication of oncogenic virus, or that ANA might be a marker of some genetic factor which predisposes to the development of adenocarcinoma of the bronchus.

Dettlaf, Bowszye and Wiecki (1973) also made a passing reference to ANA production in bronchial carcinoma.

There have been several reports of an increased prevalence of ANA in various other types of malignant disease including juvenile myelo-monocytic leukaemia (Cannat and Seligmann, 1973), carcinoma of the cervix and uterus (Nelson, 1974) and carcinoma of the prostate (Ablin, 1972).

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN CHRONIC BRONCHITIS AND SMOKING.

It was of some importance to consider the possibility of an increased prevalence of ANA's and other autoantibodies in chronic bronchitis, and in smokers compared with non-smokers, because both chronic bronchitis and the habit of smoking commonly co-exist in patients with many of the lung diseases we are considering.

Chronic Bronchitis

There is some evidence in the literature of a slight increase in the prevalence of autoantibodies including RF's and ANA's in patients with chronic bronchitis especially those with persistent infected bronchitis. Bonoma, Gillardi and Tursi (1966) studied 105 patients with chronic bronchitis and found ANA's in 18.9% and also found RF's in 61.9% using human F11 coated latex particles, 40.1% using human

F11 coated sheep cells, but only 18.3% using the Rose-Waaler test with rabbit globulin coated sheep cells. A marked reduction in the incidence of RF was observed after infection had been controlled by antibiotic therapy suggesting that the antigenic stimulus was related to the infective process.

Hodson and Turner-Warwick (1976) have also found ANA's in 14 (18%) of a group of 78 patients with chronic bronchitis. However, the 28 patients with simple smoking-related non-infected bronchitis had no evidence of ANA (0 out of 28), and the antibody was only seen in the patients with chronic infected bronchitis (at least 6 infective episodes per year), where the prevalence was 28% (14 out of 50) in comparison with 4% (2 out of 50) in a group of age and sex matched normal controls. ANA production as well as RF production in chronic bronchitis, therefore, is possibly a consequence of infection rather than the bronchitic process.

Smoking

A slight increase in the prevalence of ANA's and other autoantibodies has been reported in cigarette smokers (Mathews, et al, 1973). 3,407 normal adults were studied and ANA's were slightly more common than expected in male and female cigarette smokers of all ages. Overall, the prevalence of ANA in smokers was 8.3% compared with 7.7% in non-smokers.

Conclusion: In conclusion ANA production may relate to both chronic infected bronchitis, and to cigarette smoking but the prevalence of ANA reported in these situations is not sufficiently high to account for the much greater increases in ANA prevalence which have been reported in the lung diseases included in this study.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN LUNG DISEASE CAUSED BY INFECTIVE AGENTS

In addition to the increased prevalence of ANA and other autoantibodies in infected chronic bronchitics (Hodson and Turner-Warwick, 1976) increases in the prevalence of autoantibodies have been reported in pulmonary tuberculosis and in pneumonia relating to Mycoplasma pneumoniae infection.

There have been several reports of ANA's in pulmonary tuberculosis, but it is not yet clear whether they arise only as a consequence of drugs used therapeutically or whether in some instances they are induced by the infective process. Cannat and Seligmann (1966) reported ANA in 19% of patients with pulmonary tuberculosis who had been treated for prolonged periods with izoniazid, but showed that the prevalence was not strikingly increased in untreated patients (2%) or healthy controls (2%) matched for age and sex. Lindquist, Coleman and Osterland (1970) found ANA in up to 46.6% of patients with pulmonary tuberculosis but were unable to find any clear correlation with drug therapy. Other reports of ANA in pulmonary tuberculosis include those of Nozzoli and Lamana (1966) and Belli (1969). Luciano and Roth-

field (1973) described speckled immunofluorescent ANA in 8 out of 60 patients with pulmonary tuberculosis, although DNA binding assay in this group did not exceed that in a normal population of 90 control persons. Details of drug therapy in these patients was not given.

There has been one report of ANA occurring in 10 (25%) of 39 patients with Mycoplasma pneumoniae infection (Turner-Warwick, 1974b).

These reports suggest that in some instances ANA might be stimulated as a result of infective processes in the lung.

IMMUNE RESPONSES TO NUCLEAR ANTIGENS IN DRUG-INDUCED LUNG DISORDERS

ANA production is associated with quite a large number of drugs used therapeutically, some of which may also induce a syndrome resembling SLE. A recent review is that of Alarcón-Segovia (1969).

Procainamide can induce a lupus like syndrome which has a higher incidence of pleurisy as an initial manifestation and a lower incidence of renal involvement than is usual in the spontaneously occurring form of SLE (Blomgren, Condemi and Vaughan, 1972). The features of procainamide induced lupus are remarkably similar to the features of a group of patients with SLE who presented at the Brompton Hospital with pleuropulmonary symptoms recently reviewed by Holgate et al (1976).

Low levels of DNA binding have been reported in procainamide-induced lupus (Hughes, 1971; Winfield and Davis, 1974) and suggest slight differences in the mechanisms leading to ANA production in these situations compared with most other groups of spontaneous SLE.

Isoniazid can also sometimes induce a lupus-like syndrome in keeping with the reports of an association between ANA production and drug therapy in pulmonary tuberculosis.

Drugs which are known to induce changes in the lungs by mechanisms which probably involve hypersensitivity reactions are nitrofurantoin which can cause a predominant eosinophilic infiltration (Nicklaus and Snyder, 1968), and hexamethonium (Heard, 1962) and busulphan (Littler and Ogilvie, 1970) both of which can lead to pulmonary oedema which sometimes persists leading to fibrosis. Paraquat poisoning also gives rise to a most acute inter-stitial pulmonary fibrosis (Lanzinger, Ritz and Franz, 1969). However, there is no information in the literature of ANA production in relation to treatment with these drugs.

In view of the large number of drugs able to stimulate ANA production in predisposed individuals, it is of obvious importance to consider the possible influence of drugs in situations where ANA is demonstrated.

Drug induced ANA's are usually transient, disappearing on withdrawal of the drug, and contrasting with the persistant ANA's in various connective tissue disorders, and in patients with lung diseases followed over many years (Turner-Warwick, 1974a).

SUMMARY

Table 1 summarises the literature which has indicated an increased prevalence of immunofluorescent ANA in certain lung diseases.

There are at least two possible reasons for variable results. These are differences in diagnostic criteria for acceptance of patients, and the many different modifications of the indirect immunofluorescent staining method of Weller and Coons (1954).

The ANA prevalence is reliant upon such factors as antigenic substrate and screening dilution of test serum chosen for acceptance of positive results (Beck, 1963). Tests in our own laboratory are performed under standard conditions and reactions observed at a serum dilution of 1 in 10 or greater accepted as positive to enable meaningful comparison of our own results in different clinical situations.

TABLE 1

Summary of literature indicating an increased prevalence of immunofluorescent ANA in certain human lung diseases.

Disease	ANA prevalence (%)	Reference
CFA	28-41	Turner-Warwick & Doniach, 1965; Mackay & Ritchie, 1965; Nagaya, et al., 1969; Stack, et al., 1972; Turner-Warwick, 1974a.
Asbestosis	25-50	Turner-Warwick & Parkes, 1970; Turner-Warwick & Haslam, 1971; Kang, et al., 1973; Lange, et al., 1974.
Coal pneumoconiosis	17-34	Lipmann, et al., 1973; Soutar, et al., 1974.
Silicosis	26-36	Kang, et al., 1973; Jones, et al., 1976.
Intrinsic asthma	16-21	Hall, et al., 1966; Chackaj, et al., 1973; Turner-Warwick, 1974b.
Adenocarcinoma of bronchus	31	Hodson & Turner-Warwick, 1975.
Chronic bronchitis	0-28	Bonomo, et al., 1966; Hodson & Turner-Warwick, 1976.
Pulmonary tuberculosis	2-47	Cannat & Seligmann, 1966; Lindquist, et al., 1970.
Pneumonia (Mycoplasma pneumoniae)	25	Turner-Warwick, 1974b.

III. MAIN CONCLUSIONS FROM THE LITERATURE REVIEW

In the light of this review, the present study was undertaken to define the characteristics of ANA's in lung disease in greater detail, and to determine whether nuclear antigens might potentially stimulate cellular as well as antibody-mediated hypersensitivity reactions in the lung. The study was undertaken for the following reasons:

1. Because the evidence of increased prevalence of ANA in some lung diseases is now substantial.
2. Because the evidence suggests that this is unlikely to be a consequence of co-existent chronic infective bronchitis or smoking.
3. Because further evidence suggests that it is not a general consequence of all types of chronic lung destruction; nor on the other hand does it appear to relate to one particular type of histopathological abnormality.
4. Because there is some evidence of differences in the characteristics of the ANA's in the various lung diseases, suggesting that they might play different roles under different circumstances.
5. Because preliminary evidence suggests that ANA may participate in tissue damaging Type III immune complex mediated hypersensitivity reactions in some patients with CFA, and that in the inorganic dust diseases ANA might act as an aggravating factor stimulating particularly severe fibrotic lung damage.

6. Finally, because no-one so far has attempted to investigate the possible contribution of cell-mediated hypersensitivity reactions to nuclear antigens in these lung disorders despite the evidence that such reactions can be stimulated in some patients with SLE.

CHAPTER 2

PATIENTS AND CONTROLS

1. PATIENTS AND CONTROLS USED TO STUDY PREVALENCE OF IMMUNO-FLUORESCENT ANA

Blood from the following patients and controls was obtained by venepuncture, the serum recovered and stored without preservative at -20°C until required. Sera were collected over an 8 year period from 1965 to 1973.

PATIENTS WITH LUNG DISEASE

The majority of these patient groups have been the subject of previous studies published from the Department of Medicine, Cardiothoracic Institute, Fulham Road, London, S.W.3.

Pleuropulmonary SLE

There were 28 patients, 7 males and 21 females, in this special clinical subgroup within the general definition of SLE, who had a predominant intrathoracic disorder with respiratory symptoms the presenting feature in most cases, and who rarely had evidence of renal involvement. The clinical features have been described in detail elsewhere (Holgate et al, 1976).

All 28 met the Medical Research Council's criteria for diagnosis of SLE (MRC, 1961) and 22 also fulfilled the American Rheumatism Association's criteria (Cohen and Canoso, 1972).

All, by definition, had immunofluorescent antinuclear antibody (ANA) of titre 1 in 10 or greater, but the group was included here for the comparison. LE cells had been demonstrated on at least two occasions in all but three patients.

Pleurisy was the most frequent respiratory symptom occurring in 24 (87%) of the patients, small pleural effusions were seen in 9 (33%) and pericarditis in 15 (55%). There were 2 patients with biopsy evidence of fibrosing alveolitis.

Cryptogenic Fibrosing Alveolitis (CFA)

There were 142 patients, 86 males and 56 females. The group has been described previously (Turner-Warwick and Haslam, 1971).

All had radiographic evidence of persisting bilateral widespread pulmonary shadowing, sometimes with honeycombing and frequently denser at the bases of the lungs, and lung physiology showed a restrictive ventilatory defect.

Clinically all were breathless and had numerous basal crepitations, and 57% had finger clubbing. All other possible causes such as sarcoidosis, extrinsic allergic alveolitis and pneumoconiosis had been reasonably excluded. The diagnosis was confirmed by histological examination of lung tissue in 46.

There were 59 (42%) patients with systemic manifestations in addition to the lung lesions. These included rheumatoid disease in 36, liver disease in 16, thyroid disease in 2, Raynaud's syndrome in 3, ulcerative colitis in 3, Sjogren's syndrome in 3, renal disease in 2, scleroderma in 1, pernicious anaemia in 1 and polymyositis in 1.

Asbestosis

There were 74 patients, 66 males and 8 females. The group has been described previously (Turner-Warwick and Haslam, 1971). All had been exposed industrially to asbestos dust for periods ranging from 1 to 40 years (mean 18 years). All had severe disease with radiographic evidence of persisting bilateral widespread pulmonary shadowing showing features compatible with the diagnosis. In addition lung physiology showed a restrictive ventilative defect. Clinically all had rales on auscultation and 42 (57%) had finger clubbing.

There were 11 (15%) with evidence of associated rheumatoid disease.

Sandblaster's Silicosis

There were 30 patients, 29 males and one female. The group has been described in detail previously (Jones et al, 1976). All had been exposed to crystalline silica dust while sandblasting for periods ranging from 3 to 32 years (mean 13 years). All had radiographic pulmonary shadowing compatible with the diagnosis showing either simple nodular silicosis, or widespread conglomerate lesions.

Coal Miners' Pneumoconiosis

There were 109 patients, all males. The group has been described previously (Soutar et al, 1974). All were miners or ex-miners with a history of exposure to coal dust, and all had been accepted by the Pneumoconiosis Medical Panel as having radiographic and functional abnormalities consistent with a diagnosis of coal pneumoconiosis. The radiographic abnormalities ranged from mild simple pneumoconiosis to advanced progressive massive fibrosis.

Acute Extrinsic Allergic Alveolitis (Acute EAA)

There were 20 patients, 12 males and 8 females. The group included 13 patients with acute bird-fanciers' lung and 7 patients with acute farmers' lung.

All had radiographic evidence of nodular shadowing characteristic of acute granulomatous disease, and none had evidence of widespread irreversible lung fibrosis.

The 13 patients with acute bird-fanciers' lung (7 males and 6 females) had all been exposed to pigeons or budgerigars for varying periods from 1 to 38 years (mean exposure 12 years). In addition, all had circulating precipitating antibody to avian serum proteins. Skin prick tests with avian protein were positive in only 2 patients, but all gave positive immediate skin reactions in intracutaneous skin tests, and 12 also showed late reactions.

The 7 patients with acute farmers' lung (5 males and 2 females) had all been exposed to mouldy hay, and all had circulating precipitating antibody to Micropolyspora faeni. Skin tests were not done.

Chronic Extrinsic Allergic Alveolitis (Chronic EAA)

There were 34 patients, 24 males and 10 females. The group has been previously described (Turner-Warwick and Haslam, 1971).

All had radiographic evidence of persisting widespread pulmonary shadowing, and cases with transient nodular shadowing, reflecting more acute granulomatous lesions, were excluded. The group included 19 patients (11 males and 8 females) with chronic bird-fanciers' lung and 15 patients (13 men and 2 women) with chronic farmers' lung.

The 19 patients with chronic bird-fanciers' lung had been exposed to pigeons or budgerigars for varying periods of from 1 to 54 years (mean 19 years). All had circulating precipitating antibodies to avian serum protein. Prick and intracutaneous skin tests with avian serum protein on 14 patients showed positive immediate prick reactions in 6, positive immediate intracutaneous test reactions in 13, and positive late intracutaneous reactions in 11. In 3 other patients skin prick tests were negative.

The 15 patients with chronic farmers' lung had been exposed occupationally to mouldy hay. All had circulating precipitating antibodies to Micropolyspora faeni, but skin tests were not done.

Chronic Pulmonary Sarcoidosis

There were 33 patients, 15 males and 18 females. All had radiographic evidence of persisting bilateral widespread pulmonary shadowing, which had remained stationary or progressed despite steroid therapy over a 2 year period of assessment. Cases with acute reversible granulomatous disease, characterised by nodular lesions on chest radiographs, were excluded.

There was histological confirmation of the diagnosis in all patients, either by Kveim biopsy (22), mediastinal node biopsy (16) or lung biopsy (2). In addition the Mantoux skin test with PPD was negative in all but 3 patients.

Chronic Bronchitis

There were 37 patients, 27 males and 10 females.

The diagnostic criteria were those of the Medical Research Council (1965), the main criterion being chronic cough and sputum on most days during at least three consecutive months for at least two successive years.

All the patients had radiographic evidence of lung damage relating to the bronchi, and lung physiology showed a chronic obstructive ventilatory defect. Six patients only had evidence of repeated chest infection with purulent sputum.

Cases associated with bronchial asthma, characterised by wheeze and blood eosinophilia, were excluded.

Intrinsic Asthma

There were 48 patients, 17 males and 31 females. The group has been previously described (Turner-Warwick and Haslam, 1970).

All had predominant wheeze and breathlessness with reversible airways obstruction and minimal cough and sputum. Lung radiographs were normal. No known external causal allergens could be identified, and skin tests to a standard range of 23 common allergens were negative. All had shown an absolute blood eosinophilia of over 500 mm^3 on at least one occasion.

Extrinsic Asthma

There were 43 patients, 18 males and 25 females.

All had predominant wheeze and breathlessness, reversible airways obstruction, minimal cough and sputum, blood eosinophilia, and normal lung radiographs.

There was evidence of multiple extrinsic causal allergens in all the patients, with positive skin prick test reactions to several of 23 standard common prick test allergens, but none of the patients had serum precipitins to extracts of the allergens.

Seven patients had associated eczema and 12 rhinitis. Cases with associated bronchitis were excluded.

PATIENT AGE

The mean age and age distribution (mean age $\pm$ 2SD) of the total, and male and female patients in each of the above lung disease groups is shown in Table 2.

This information was used to select normal control groups, of similar mean age and age range (within 5 years), for comparison with the patient groups.

CONTROLS WITHOUT LUNG DISEASE

Asbestos Exposure

This was a special control group selected for comparison with the asbestosis disease group. It has been previously described (Turner-Warwick and Haslam, 1971).

There were 74 workers, 72 males and 2 females, matched for age and duration of exposure to asbestos dust (within 5 years) with the patients in the asbestosis disease group but in whom the chest radiographs showed no evidence of intra-pulmonary abnormalities and in whom no finger clubbing was observed. Scanty rales were audible in 13 subjects from this group.

TABLE 2

The numbers and age distributions (Mean age in years ± 2SD) of the patients in each lung disease group.

Disease	Total patients			Male patients only			Female patients only		
	No.	Mean age	Age range (Mean age ± 2SD)	No.	Mean age	Age range (Mean age ± 2SD)	No.	Mean age	Age range (Mean age ± 2SD)
Pleuropulmonary SLE	28	50	21-78	7	53	39-68	21	49	17-80
CFA	142	56	30-81	86	55	31-80	56	57	29-84
Asbestosis	74	54	38-71	66	54	37-71	8	60	51-68
Asbestos exposure	74	53	36-71	72	53	36-71	2	58	47-69
Sandblaster's silicosis	30	46	26-65	29	46	26-65	1	41	0
Coal miner's pneumoconiosis	109	61	43-79	109	61	43-79	0	0	0
Acute EAA	20	38	6-70	12	29	8-50	8	52	29-75
Chronic EAA	34	54	36-72	24	54	37-72	10	54	35-74
Chronic sarcoidosis	33	37	9-65	15	36	6-67	18	36	11-61
Chronic bronchitis	37	61	36-89	27	65	48-81	10	52	15-89
Intrinsic asthma	48	48	18-70	-	-	-	-	-	-
Extrinsic asthma	43	33	1-64	18	29	2-60	25	36	4-67

- = Not known

Normal Healthy Controls

There were 205 normal healthy persons, 121 males and 84 females, without evidence of lung or other disease.

The majority of age 20 to 65 years were blood donors, and a number were volunteer medical and laboratory staff. Volunteers over 65 years of age were kindly referred by a general practitioner colleague.

II. PATIENTS AND CONTROLS USED TO DEFINE THE CHARACTERISTICS OF ANA

The following patients and controls all had positive serum ANA of titre 1 in 10 or greater by immunofluorescence.

Serum from these patients and controls was collected and stored at -20°C until required.

PATIENTS WITH LUNG DISEASE

The diagnostic criteria were those described above (I.).

Pleuropulmonary SLE

There were 28 patients, 7 males and 21 females, and the group is the same as that described above (I.).

The mean age of the patients was 50 years, SD 14 years.

Cryptogenic Fibrosing Alveolitis (CFA)

There were 76 patients, 39 males and 37 females. Thirty six had systemic manifestations in addition to the lung changes. The mean age of the patients was 57 years, SD 13 years.

Asbestosis

There were 33 patients, 30 males and 3 females. The duration of exposure to asbestos dust ranged from 1 to 40 years, mean 20 years. The mean age of the patients was 57 years, SD 8 years.

Sandblaster's Silicosis

There were 22 patients, 21 males and 1 female. The duration of exposure to silica dust ranged from 6 to 28 years, mean 13 years. The mean age of the patients was 47 years, SD 11 years.

Intrinsic Asthma

There were nine patients, 5 males and 4 females. The mean age of the patients was 45 years, SD 13 years.

CONTROLS WITHOUT LUNG DISEASE

There were 10 healthy normal persons, 5 males and 5 females. The mean age was 59 years, SD 13 years.

III. PATIENTS AND CONTROLS USED TO STUDY LYMPHOCYTES

Peripheral blood leucocytes and time-matched serum samples were collected from the following patients and controls.

PATIENTS

Forty nine patients were studied in whom ANA had been detected by a standard immunofluorescent staining test in serum dilutions of one in ten or greater. The clinical groups are set out in Table 3. The diagnostic criteria were as follows:

Pleuropulmonary SLE

Nine patients were selected having multisystem disease including pleuropulmonary involvement fulfilling the criteria suggested by the American Rheumatism Association (Cohen and Canoso, 1972), and in whom LE cells had been detected. Two were receiving corticosteroid therapy at the time of testing, and two others were studied before and during treatment.

Cryptogenic Fibrosing Alveolitis (CFA)

Twenty patients were selected, the diagnostic criteria being those described previously. (I. above). The diagnosis was confirmed in six instances by lung biopsy. Eight were not receiving corticosteroid therapy at the time of testing. Nine of the twenty were receiving corticosteroid therapy at the time of testing and three others were studied before and during treatment.

TABLE 3

Total number of patient and control leucocyte migration studies.

Disease	Total	Untreated	Treated
Pleuropulmonary SLE	11	7	4
CFA	23	11	12
CFA - with systemic sclerosis	3	3	0
Asbestosis	10	9	1
Intrinsic asthma	9	7	2
Sjögren's syndrome	1	1	0
Controls	16	16	0
Totals	70	51	19

Asbestosis

Ten patients were included in whom there was a history of asbestos exposure, persisting bilateral radiographic shadowing and in whom crepitations were heard on auscultation. One was receiving corticosteroids.

Intrinsic Asthma

Nine patients were selected with reversible airways obstruction in whom the clinical history failed to reveal external allergens and in whom immediate prick test responses to a standard range of twenty-three antigens were negative. Two were receiving corticosteroid therapy.

Systemic Sclerosis

The three patients studied had evidence of widespread pulmonary disease with additional skin changes typical of scleroderma.

Sjogren's Syndrome

One patient with grossly deficient secretions but without pulmonary involvement was studied.

CONTROLS

Sixteen healthy volunteer subjects, in whom ANA was not detectable in a one in ten serum dilution, were used as controls.

CHAPTER 3

MATERIALS AND METHODS

I. ANTISERA FOR IMMUNOFLUORESCENT STAINING TESTS

COMMERCIAL SOURCES

Sheep antihuman immunoglobulin conjugated with fluorescein iso thiocyanate, was obtained from Wellcome Reagents Ltd. The antiserum was polyvalent reacting with human IgG, IgM and IgA.

Goat antihuman IgG, goat antihuman IgM, and goat antihuman IgA were unconjugated antisera obtained from the Hyland division of Baxter Laboratories.

Horse antihuman complement components C3 and C4 (Pondman, Es and Schuurs 1965), was an unconjugated antiserum obtained from the Blood Group Reference Laboratory, Amsterdam.

CONJUGATION OF ANTISERA WITH FLUORESCEIN ISOTHIOCYANATE (FITC)

FITC-conjugated antihuman IgG, IgM, IgA and complement (C3 and C4) were prepared from the commercial unconjugated antisera, adhering as closely as possible to the recommendations advised by Johnson and Holborow(1973). Reasonably large batches of antisera were conjugated so that comparative studies could be completed with the same batch.

The antisera all had at least 16 to 32 precipitating units of anti-globulin activity per ml (Johnson and Holborow, 1973), and only the globulin fractions were conjugated to avoid problems of non-specific staining, which can be caused by conjugated albumin and other irrelevant serum proteins.

The FITC-conjugation procedure was a modification of the method of Marshall, Eveland and Smith (1958).

1 volume of antiserum was diluted to 10 volumes with cold ($4^{o}C$) 0.15M phosphate buffered saline pH 7.0, and an equal volume of cold ($4^{o}C$) saturated ammonium sulphate solution added slowly with mixing to precipitate the antiserum globulins. The solution was left for an hour at $4^{o}C$ and the precipitate recovered by centrifugation at 17,000 rpm for 15 minutes at $4^{o}C$. It was redissolved to the original 1 in 10 volume with phosphate buffered saline and purified by re-precipitation.

The purified globulins were redissolved in a minimum volume of unbuffered 0.15M saline, and freed from ammonium sulphate by gel filtration through a column of Sephadex G25 (Pharmacia Ltd., Uppsala, Sweden) equilibrated with 0.15M unbuffered saline.

The protein content of the eluted globulin fraction was measured by comparison with a Behringwerke protein standard using the Folin-Ciocalteau Reagent (Lowry, et al 1959) and reading absorption at 750 mμ in a spectrophotometer.

The solution was diluted to a protein concentration of 10 mg/ml with 0.15M unbuffered saline including a final 10% concentration by volume of 0.5M sodium carbonate/bicarbonate buffer, pH 9.0, then cooled to 4°C. 0.03 mg of the anhydrous pure Isomer I of fluorescein isothiocyanate (Sigma Chemical Co. Ltd.) was weighed out for each 1 mg of protein globulin, mixed with a small quantity of Whatman CF11 cellulose powder to aid dispersion of the dye, then thrown into the chilled globulin solution. Conjugation was allowed to proceed for 18 hours at 4°C, with continuous gentle agitation on a haematocrit rotator.

After conjugation, the cellulose powder was removed by centrifugation, and the supernatant (containing FITC-conjugated globulins) freed from excess unconjugated FITC by gel filtration at 4°C through a column of Sephadex G25 equilibrated with 0.15M phosphate buffered saline. The eluted conjugate was collected in serial 1 ml fractions and the most concentrated fractions pooled.

Finally, the protein concentration of the conjugate was measured, and an estimate of the FITC concentration obtained by diluting the conjugate approximately 1 in 10 (to achieve a suitable working range for spectrophotometric reading), reading its absorption in the spectrophotometer at 492 mμ (the wavelength of maximum absorption of FITC) and correcting for irrelevant absorption not attributable to FITC by subtraction of half the absorption at 320 mμ.

The fluorescein: protein ratio was determined from the following expression which was derived by Holborow and Johnson (1967):

$$F : P = \frac{2.06 \times E492\ m\mu\ (\text{corrected}) \times \text{Dilution}}{\text{mg/ml protein}}$$

The F : P ratios of the FITC conjugates prepared for use in this study were all between 4.8 : 1 and 5 : 1.

Conjugates were stored in aliquots at $4^{o}C$.

TISSUE POWDER ABSORPTION OF FITC-CONJUGATES

All FITC-conjugates used in the present study were absorbed immediately prior to use with tissue powder as recommended by Coons and Kaplan (1950) to remove conjugated globulins of particularly high net negative charge which are primarily responsible for non-specific staining (Curtain, 1958). For absorption, 1 volume of moist pig liver powder (acetone-dried; Wellcome Reagents Ltd.) was added to 2 volumes of FITC-conjugate and the mixture agitated for 1 hour at room temperature on a rotary mixer.

After absorption the conjugate was freed from tissue powder by filtration through a 5μ pore size Millipore membrane in a Hemmings' filter.

It was found unadvisable to re-use tissue powder absorbed conjugates after storage.

ABSORPTION OF FITC-CONJUGATED HORSE ANTIHUMAN COMPLEMENT (C3 AND C4)

FITC-conjugates of antihuman complement were absorbed with pure human IgG before use as recommended by Lachmann et al (1962) to remove any traces of antihuman IgG.

For the absorption, an amount of pure human IgG equivalent to double the antiserum protein was added, the mixture incubated for an hour at room temperature, then centrifuged to remove any precipitated complexes.

CONFIRMATION OF SPECIFICITY AND STANDARDIZATION OF FITC-CONJUGATED ANTISERA

Immunoelectrophoretic analysis, using a modification of the micromethod of Scheidegger (1955) with fresh human serum as the antigen, confirmed that the FITC-conjugated antisera used in this study all gave characteristic arcs of immunoprecipitation with no evidence of other lines indicating lack of specificity.

Ouchterlony double-diffusion used as suggested by Holborow and Johnson (1967) with fresh human serum as the antigen, confirmed that Kappa and Lamda light chain cross-reactivity had been removed from FITC-conjugates of antihuman IgG, IgM and IgA.

The optimum working dilution of each FITC-conjugated antihuman immunoglobulin antiserum was determined using an indirect immunofluorescent staining test for ANA (P. 85) and standard positive control sera with ANA of known titre (P. 90) with respect to summated immunoglobulin classes and individual immunoglobulin classes of ANA. The highest dilution of each conjugate achieving the standard maximum titre of ANA with the maximum definition of specific staining and minimal non-specific background staining was selected for use.

For FITC-conjugated antihuman complement, the activity was confirmed and the working dilution determined similarly using an indirect staining method (p. 91) and sera with known titres of complement-fixing ANA.

11. NUCLEASE ENZYMES

NUCLEASE S1

The pure Nuclease S1 used in this study was a gift from Dr. R. I. Kamen, Imperial Cancer Research Fund.

It is an enzyme from Aspergillus oryzae which can specifically digest non-base-paired nucleic acid single-strands and loops by splitting the phosphodiester bonds linking the nucleotide chains. (Ando, 1966).

RIBONUCLEASE

Ribonuclease A from bovine pancreas, was obtained from Boehringer Mannheim (Code 15331, batch 7422325).

It is an endonuclease which cleaves RNA but not DNA, releasing pyrimidine-3'-phosphates and oligonucleotides with pyrimidine-3'-phosphate terminal groups. Its optimal stability is pH 2.0-5.0 and optimum activity pH 7.7.

It was stored in freeze dried form at +4^{o}C until used.

DEOXYRIBONUCLEASE

Deoxyribonuclease 1 from bovine pancreas, was obtained from Miles-

Seravac Laboratories (Code 36-152, batch 1355A).

It is an endonuclease which depolymerizes DNA, releasing mainly 5'-monoesters. Its optimal stability is pH 5.0 and optimal activity pH 6.0 - 7.0, and bivalent cations (e.g. Mg^{++}) are obligatory.

It was stored in freeze dried form at $-20^{o}C$ before use.

111. NUCLEAR ANTIGENS

Single batches of all nuclear antigens, with the exception of frozen rat liver sections, were used for the whole series of in vitro antibody and lymphocyte studies in order to make meaningful comparisons.

Rat Liver

This was used as a source of cell nuclei for a number of indirect immunofluorescent staining tests (p 85 ;91 ;107).

Young rats were killed as required, the livers removed with a minimum delay, cut into blocks 1 cm^3 and snap frozen on strips of copper foil pre-cooled on solid carbon dioxide. The frozen tissue blocks were used immediately, or stored in an air tight container at $-70^{\circ}C$ and used within 5 days, after which time controls indicated a loss of antigenicity.

For indirect immunofluorescent tests, sections 2 to 4 μ thick were cut using a Bright's cryostat, mounted in pairs or triplicates on clean grease-free microscope slides, air dried for 30 minutes, then used directly.

Calf-Thymus Deoxyribonucleoprotein

This was prepared by the method of Johns et al, (1960), and kindly supplied by Dr. E. W. Johns (Chester Beatty Institute). It was

stored as at 1 mg/ml suspension in ethanol at -20°C.

For use in immunofluorescent tests for deoxyribonucleoprotein antibodies (p 94), drops of the stock 1 mg/ml ethanolic suspension were applied to clean, grease-free microscope slides (2 drops per slide), allowed to air dry for 30 minutes, then used directly.

Commercial Calf-Thymus 'Double-stranded' Deoxyribonucleic Acid ('DS'-DNA)

This was prepared by a modification of the method of Zamenhof (1958), and was obtained in a salt-free freeze dried form from Miles-Seravac Laboratories (Code 36-155; Batch 22B). It was stated to be a highly polymerized material, with a DNA content of 83.8%, ribonucleic acid less than 1%, protein 0.23% and moisture 9.4%.

For use in Ouchterlony double-diffusion tests (p.95) the calf-thymus 'DS'-DNA was dissolved slowly over a period of 24 hours at room temperature in 0.01M K_2HPO_4/KH_2PO_4 buffer, pH 7.2, at a concentration of 1 mg/ml, avoiding harsh treatment such as mechanical stirring which might cause strand separation.

Assay of a 5.0 mg/ml solution of this DNA in 0.1M sodium citrate, pH 7.4, and of a similar solution after heat treatment at 76°C for 1 hour in a Ferranti-Shirley cone and plate viscometer at 25°C applying a range of shear rates from 0 to 18,000 S^{-1}, demonstrated that the heat treatment effected a percentage decrease in specific

viscosity at all shear rates varying from 43% to 53%. A percentage decrease of greater than 2% suggests that the original material was probably damaged (Zamenhof, 1958), either with free single strands or strand shearing. Heating at boiling point in a water bath for 10 minutes then rapid cooling on ice was found to effect a similar decrease in specific viscosity from 34% to 38%.

Dr. K. Shooter (Chester Beatty Research Institute) has recently obtained further evidence of single-strand breaks in this DNA antigen from measurements of the sedimentation coefficients at neutral pH and in alkali, using a Beckmann-Spinco analytical ultracentrifuge fitted with the ultra-violet absorption optical system (Haslam et al, 1976).

Nuclease SI 'Purified' 'Double-stranded' Calf-Thymus DNA

An attempt was made to reduce, or remove, single-strand contamination from the commercial calf-thymus 'DS'-DNA using the enzyme Nuclease S1 (p. 73) and a modification of the method of Vogt (1973). A trial trace-labelling experiment was first performed, employing p^{32}-labelled single-strand DNA (a gift from Dr. R. I. Kamen) and conducted in the presence of excess calf-thymus 'DS'-DNA, which demonstrated the total digestion of 2 ml of a 1 mg/ml solucion of heat denatured single-strand calf-thymus DNA using 1.5 μl of the Nuclease SI solution in an optimum time of 30 minutes, at a temperature of 50°C.

For the actual purification 6 ml of a 1 mg/ml solution of commercial calf-thymus 'DS'-DNA was prepared in a buffer consisting of 0.05M potassium acetate, pH 4.5, 0.2M sodium chloride and 1 mM Zinc sulphate. 6 µl of 2-mercaptoethanol (7 mM/ml) was added, and finally 4.5 µl of Nuclease SI solution. After mixing, the solution was incubated at 50°C in a water bath for 30 minutes and shaken gently at regular intervals. The reaction was terminated by immediate cooling in an ice bath, and the 'purified' 'double-stranded' DNA recovered by chloroform-phenol extraction as follows: The reaction solution was vortexed with an equal volume of a 1:1 mixture of chloroform and redistilled phenol, and the phases were separated by centrifugation at 5,000 rpm for 10 minutes at room temperature. The upper aqueous phase containing the 'purified' 'double-stranded' DNA was recovered and re-extracted with chloroform-phenol, then extracted twice more with chloroform containing 1% isoamyl alcohol. The interphase was at this stage perfectly clear, indicating the complete removal of digested fragments and enzymic protein. Traces of phenol were finally removed by exhaustive dialysis against a buffer of 0.01M Tris (Trihydroxyaminomethane) with 1M NaCl, pH 7.5.

The solution of Nuclease SI 'purified' 'double-stranded' calf-thymus DNA was stored briefly at 4°C then dialysed into 0.01M K_2HPO_4/KH_2PO_4 buffer, pH 7.2, for immediate use in Ouchterlony double-diffusion tests.

Calf-Thymus Single-stranded Deoxyribonucleic Acid (SS-DNA)

This was prepared for use in Ouchterlony double-diffusion tests (p. 96) from a 1 mg/ml solution of calf-thymus 'DS'-DNA in 0.01M K_2HPO_4/KH_2PO_4 buffer, pH 7.2. Single-stranding was achieved by heating the solution in a boiling water bath for 10 minutes, shaking quickly to disperse the strands, then rapid immediate cooling in an ice bath. The solution was subsequently kept at temperatures below 15°C prior to, and during, test procedures to avoid spontaneous strand recoil.

I^{125}-labelled 'Double-stranded' HeLa Cell Deoxyribonucleic Acid

This was used as the antigen for Farr-binding tests (p.104), and was a gift from Dr. John Holian of the Radiochemical Centre, Amersham.

The radioactive label was introduced as an internal label into HeLa cell DNA by culturing the cells in medium containing I^{125} iodo-deoxyuridine (Glass et al, 1973), and the labelled DNA was extracted by a modification of the method of Marmur (1961).

Dr. K. Shooter (Chester Beatty Research Institute) has examined this antigen using ultracentrifugation methods and has been unable to detect any single-strand breaks. (Haslam et al, 1976).

Total Yeast Ribonucleic Acid (RNA)

This was obtained from Miles-Seravac Laboratories, Code 23-340, Lot 3. It was stated to be a relatively low molecular weight depolymerized material assaying at least 94% RNA.

The material was practically insoluble in 0.01M buffers rendering problems for its use in Ouchterlony double-diffusion tests which are normally carried out at this low molarity with macro-molecules such as nucleic acids to aid their diffusion (Clausen, 1972).

Ouchterlony double-diffusion tests were, however, attempted (p.85) using 5 mg/ml and 1 mg/ml solutions of RNA in three alternative buffers: 0.1M sodium acetate/acetic acid buffer, pH 6.5; 0.1M K_2HPO_4/KH_2PO_4 buffer, pH 7.2 and 0.14M NaCl/0.015M sodium citrate buffer, pH 7.1.

For use in counter-current electrophoresis electroimmunoprecipitation (p.105), 200, 100 and 20 µg/ml solutions of RNA were prepared in 0.05M sodium barbitone/HCl buffer, pH 8.2.

NUCLEAR ANTIGENS FOR LYMPHOCYTE STUDIES

The following nuclear antigens were prepared for use in leucocyte migration tests (p.108).

Rat Liver Nuclei

These were isolated from a homogenate of fresh rat liver by differential centrifugation through sucrose solutions of increasing density (Stirpe and Aldridge, 1961). The final nuclear pellet was resuspended to one tenth the original volume of homogenate with 1.6M sucrose in 0.4M $MgCl_2$, and stored at -20°C in suitable test aliquots of 0.4ml. The dry weight of nuclear material per aliquot was determined. Purity was confirmed by electron microscopy (Plate 1). For use in tests, nuclei were washed twice with Eagle's Basal Medium (EBM) (Wellcome Reagents Ltd. Code TC30), and resuspended to the required test concentration in EBM containing 20% inactivated foetal calf serum (EBMS).

Calf-Thymus Deoxyribonucleoprotein

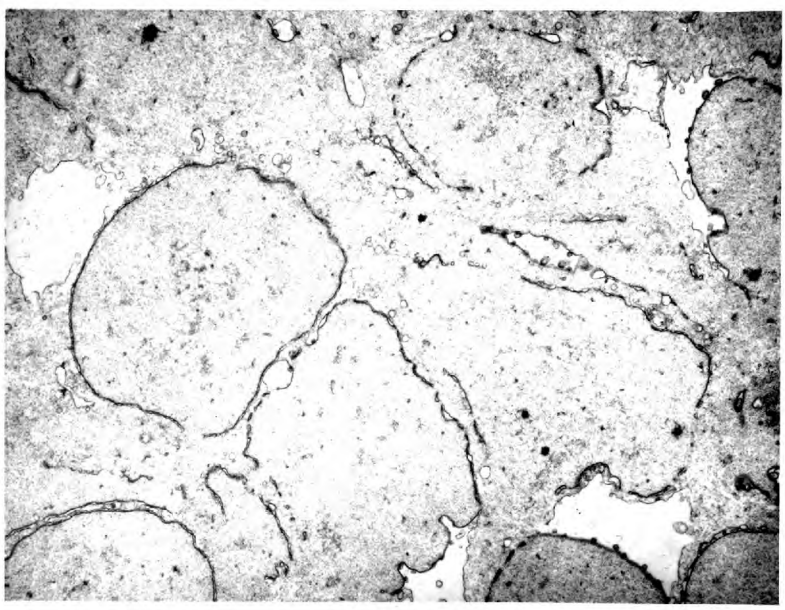
This was the same batch as that used for antibody studies (p.75).

For the lymphocyte studies, a 2 mg/ml suspension was stored in ethanol at -20°C, and for use the required amount was removed, excess ethanol decanted and replaced with an equal volume of EBMS, and final traces of ethanol removed by dialysis against EBM.

The suspension was finally adjusted to the required test concentration with EBMS.

Electron micrograph of purified rat liver cell nuclei used in peripheral leucocyte migration tests. (Magnification x 3740)

Plate I



Commercial Calf-Thymus 'Double-stranded' Deoxyribonucleic Acid ('DS'-DNA)

This was the same batch of commercial calf-thymus 'DS'-DNA as that used in the antibody studies (p.76).

For lymphocyte studies it was dissolved slowly in EBMS at the required concentration without mechanical stirring.

Calf-Thymus Single-stranded Deoxyribonucleic Acid (SS-DNA)

This was prepared from a 0.5 mg/ml solution of commercial calf-thymus 'DS'-DNA in EBM by heating in a water bath at boiling point for ten minutes, then immediately cooling in an ice-bath. The volume was adjusted with EBMS to the required test concentration and the pH corrected back to 7.2.

Total Yeast Ribonucleic Acid (RNA)

This was the same as that used for the antibody studies (p.80).

It was dissolved in EBMS at the concentration required and the pH adjusted to 7.2.

IV. METHODS TO STUDY ANTINUCLEAR ANTIBODIES

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR ANTINUCLEAR ANTIBODIES (ANA)

The method used was a modification of the indirect immunofluorescent staining technique of Weller and Coons (1954). Fresh cryostat-cut frozen sections of rat liver, 2 to 4 μ thick were used as a source of nuclear antigens. After air-drying, slides bearing tissue sections were placed in a moist chamber (multislide staining chamber, Shandon Scientific Co. Ltd.), and each section covered with a patient or control serum diluted 1 in 10 (p46) in Coon's barbitone buffer, pH 7.2 (Sodium barbitone 8.24g; sodium chloride 34g; N HCl 32.24 ml; made up to 2 litres with distilled water and diluted with an equal volume of distilled water before use). The sections were from then on kept moist, since drying results in staining artifacts.

The preparations were incubated for 20 minutes at room temperature to allow time for any serum ANA to bind to the appropriate nuclear antigens, then flushed with Coon's buffer to remove excess serum, and washed in fresh buffer for 30 minutes with gentle agitation on a Luckham's horizontal rotator. After washing, excess buffer was wiped from around each section, and the sections covered with pig-liver powder-absorbed FITC-conjugated sheep (polyvalent) antihuman immunoglobulin diluted according to a previously

determined optimum dilution factor (p. 72) for the batch of antiserum (normally 1 in 8). The sections were incubated for a further 20 minutes at room temperature to allow time for the fluorescent tracer to attach to any tissue bound human ANA immunoglobulin, then again flushed with Coon's buffer and washed in fresh buffer for 30 minutes.

Preparations were finally mounted under Chance No. 1 cover slips in a solution of equal parts glycerol and Coon's buffer, and examined under ultraviolet light using a Reichert Zetopan Research Microscope and applying darkground illumination (see below).

Positive nuclear immunofluorescence was graded in four categories, $\pm$, +, ++, or +++ to denote increasing intensity, and reactions of particular interest were recorded by photomicrography (p. 88). At least one known positive and one known negative control serum was included in every batch of tests.

The technical procedure described here in detail was the same for the various indirect-staining tests described below and will therefore not be repeated.

FLUORESCENCE MICROSCOPY

FITC-stained preparations were examined under a Reichert 'Zetopan' Research Microscope using transmitted ultraviolet light and employing

a darkground Cardioid Condenser (Reichert Neo 1.8/1.42).

The ultraviolet light source was a high pressure mercury vapour lamp, Wotan HBO 200 watt, which emits mainly in the 365 mμ and 435 mμ light wavelengths (ultraviolet blue) and is thus suitable for the excitation of FITC-conjugates which have maximum fluorescence emission at 520 mμ (apple-green).

Light of unwanted wavelengths was removed by passage through a heat-stopping filter (Schott and Genossen KG 2/2mm) and a primary exciter filter (Schott and Genossen UG 1/1.5mm) housed between the UV light source and the microscope stage. Secondary protective filters (Wratten 2B/3mm sandwiched between Schott GG 13/3mm + 1mm filters) were incorporated between the objective lens and eyepiece to absorb ultraviolet blue light. The microscope objectives were achromatic dry high power objectives giving magnifications of x 25, x 40 and x 63.

The microscope slides used for immunofluorescent preparations were 0.8 - 1.0mm thick, and cover slips were Chance No. 1 grade. Slides or co slips of greater thickness caused appreciable reduction in light transmission.

The immersion medium used between the darkground condenser and microscope slides was the same as that used to mount sections, and consisted of equal parts of glycerol and Coon's barbitone buffer, pH 7.2.

PHOTOMICROGRAPHY

Colour photomicrographs of fluorescent preparations were taken with Kodak High Speed Ektachrome film, speed ASA 160, and Wratten 2E or Wratten 2E/C005 filters (for strong or weak fluorescence respectively) were incorporated in the phototube between the objective and camera lens to achieve successful colour reproduction by correcting for the pale yellow colour of the secondary ultraviolet blue stopping filters.

Exposure times were between 2 and 3 minutes.

METHODS TO REDUCE INTER AND INTRA-OBSERVER VARIABILITY IN IMMUNOFLUORESCENT TEST READING

Inter-observer Variability

1. Immunofluorescent tests were read independently by 3 regular readers and given four grades of intensity: $\pm$, +, ++ and +++.

2. Reading grades associated with inter-observer variability $\geqslant$ 5% were considered suspect.

3. Weak $\pm$ positive readings in all the immunofluorescent methods were responsible for most disagreement and were eliminated from counts.

Intra-observer Variability

This was controlled by repeating discordant results twice, and if still disagreement twice more, then accepting a positive result only if it occurred on 3 of the 4 occasions. This method has been described in detail by Hodson and Turner-Warwick, 1975.

CONTROLS TO CONFIRM IMMUNOLOGICAL SPECIFICITY OF IMMUNOFLUORESCENT STAINING

Evidence that staining in the various indirect immunofluorescent tests was immunologically specific, was obtained from the following control observations:

1. Removal of specific staining by prior absorption of FITC-conjugated antisera with the respective antigens; e.g. the removal of specific IgG staining by prior absorption of anti-IgG with pure IgG.

2. Effective blocking of specific staining by incubating the bound antigen/antibody complex with an intermediate layer of the unconjugated antiserum; e.g. the ability of unconjugated antihuman IgG to block specific staining with FITC-conjugated antihuman IgG.

3. Failure to block specific staining by incubation of the bound antigen/antibody complex with an intermediate layer of unconjugated antiserum of a different specifity; e.g.

failure of unconjugated anti-IgM and IgA to block specific staining with FITC-conjugated anti-IgG.

4. Failure to obtain similar staining with unrelated FITC-conjugates, e.g. FITC-conjugated rabbit anti-Clostridium septicum (Wellcome Reagents Ltd.).

5. Failure to obtain similar staining when FITC-conjugated antisera were applied directly to the substrate antigen in the absence of the intermediate antibody layer.

TITRATION OF IMMUNOFLUORESCENT ANA

Immunofluorescent ANA's detected using the standard 1 in 10 dilutions of sera were quantified by titration (Friou, 1958).

Each positive serum was retested using the same indirect-staining method but in a range of different dilutions 1 in 10; 1 in 20; 1 in 40; 1 in 80; 1 in 160; 1 in 320 and 1 in 640. The ANA titre for each positive serum was the highest serum dilution to which nuclear immunofluorescence could be detected.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR IMMUNOGLOBULIN CLASSES OF ANA

The method was similar to that used by Barnett et al (1964).

Sera shown to contain ANA in the standard immunofluorescent screening test with FITC-conjugated polyvalent rabbit antihuman immunoglobulin, were retested in triplicate using the same indirect-staining method but separately with monovalent FITC-conjugated goat antihuman IgG, IgM and IgA. In this way it was possible to identify which of these three main Immunoglobulin Classes were involved in the ANA activity in each positive serum. (Tests for IgE and IgD ANA were not performed).

In addition, special attention was given to describing accurately the patterns of nuclear immunofluorescence observed in the different immunoglobulin classes.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR COMPLEMENT-FIXING ANA

The modification of the immunofluorescent indirect-staining method used to determine the complement fixing ability of tissue-bound ANA was similar to that used by Beck and Hughes (1970).

ANA positive sera were diluted 1 in 10 using 1 part ANA positive serum, 4 parts Coon's barbitone buffer and 5 parts of ANA negative

serum obtained from freshly defibrinated blood from a normal healthy volunteer (PH). The fresh human serum was used to provide a source of active human complement. The diluted sera were applied to rat liver sections as in the standard test for ANA, and the preparations incubated for 20 minutes at room temperature to allow time for ANA's to bind to the appropriate nuclear antigens, and subsequently for 30 minutes at 37°C to allow time for complement to fix to those immune-complexed ANA's with positive complement-fixing ability.

(The technical manipulations from defibrination of normal volunteer blood to application of diluted ANA positive sera to rat liver sections were accomplished within 30 minutes). The preparations were washed for 30 minutes, then incubated with pig liver powder and IgG absorbed horse antihuman complement (C3 and C4) for 30 minutes as a fluorescent tracer for fixed human complement. They were washed for a final 30 minutes, mounted and examined under ultraviolet light for evidence of positive nuclear immunofluorescence indicating complement bound to immune-complexed ANA.

In addition to the routine positive and negative controls, a control of the undiluted fresh serum used as the source of active complement was always included.

LE CELL TESTS

LE cell tests were performed as a routine diagnostic procedure for

patients with SLE and cryptogenic fibrosing alveolitis in the Department of Haematology, Brompton Hospital, and read by an experienced observer. A direct method was used employing the patient's defibrinated blood and incubating the buffy coat leucocytes and serum at 37°C for 30 minutes (Dacie and Lewis, 1970).

LE cell tests were not a routine diagnostic procedure for patients with asbestosis, silicosis and intrinsic asthma, and immunofluorescent ANA positive sera from patients with these diseases were examined for LE cell factor using an indirect method involving incubation of buffy coat leucocytes from a normal volunteer of O^- blood group with each patient's serum at 37°C for 30 minutes (Dacie and Lewis, 1970). This method was also used to study ANA positive sera from occasional patients with SLE and cryptogenic fibrosing alveolitis when the results of direct tests were for various reasons not available.

After incubation, leucocyte smears were air dried, fixed in methanol for 3 minutes, stained with May-Grunwald stain and finally examined for LE cells under a light microscope using a high power oil-immersion objective, and check read by an experienced haematologist.

A number of patients with SLE and cryptogenic fibrosing alveolitis were studied using both direct and indirect LE cell test methods and the results correlated closely.

INDIRECT IMMUNOFLUORESCENT STAINING TEST FOR ANTIBODIES TO DEOXYRIBONUCLEOPROTEIN

ANA positive sera were examined for evidence of ANA's reactive with deoxyribonucleoprotein using a modified indirect immunofluorescent staining test with calf-thymus deoxyribonucleoprotein as the antigenic substrate and similar to the deoxyribonucleoprotein 'spot' test described by Friou (1967).

Microscope slides were coated with calf-thymus deoxyribonucleoprotein as previously described (p. 75) and incubated with undiluted ANA positive sera for 30 minutes at room temperature. After washing for 1 hour, the preparations were stained with pig-liver powder absorbed FITC-conjugated sheep polyvalent antihuman immunoglobulin for 30 minutes, washed for a final 1 hour period, mounted and examined under ultraviolet light for positive immunofluorescence.

METHODS TO DETECT ANTIBODIES TO DEOXYRIBONUCLEIC ACID (DNA)

Choice of Methods

It has not yet been possible to define standard methods and antigens to study antibodies to double and single-stranded DNA (Arthritis and Rheumatism Council Workshop, 1976).

For this study, Ouchterlony double-diffusion with commercial calf-thymus DNA as antigen, was chosen as an example of one of the

earlier methods which has had wide application (Tan et al, 1966). The problem of non-immunological precipitation of DNA with serum proteins such as C1q (Agnello et al, 1969) was guarded against by methods which are described on page 96.

For comparison, a modification of the more recent Farr assay method with 'DS' I^{125} HeLa cell DNA as antigen (Glass et al, 1973), was chosen in the hope that it might demonstrate antibodies to double-stranded DNA since there was evidence that the antigen had minimal single-strand content (p.79), and clinically the test had shown diagnostic specificity for SLE (Holian et al, 1975).

Ouchterlony Double Diffusion Test for 'DS' DNA Precipitins

The Ouchterlony double diffusion test used was essentially the modification of Tan and Kunkel, 1966. The modifications are directed towards achieving conditions under which the macromolecular DNA can diffuse easily through agarose gel the main ones being reducing the concentration of the gel to 0.4%, and conducting the test in a buffer of low ionic strength.

Agarose was dissolved at 0.4% concentration in 0.01 M K_2HPO_4/KH_2PO_4 buffer, pH 7.2, using a boiling water bath to achieve solution. (The double potassium salt buffer was used in preference to a potassium/sodium salt buffer, since, unlike the latter, the pH remains stable at low temperatures with less risk of spurious or indistinct precipitation lines due to a sub-optimal pH). The molten agarose was applied in 14.5 ml volumes to 8.2 x 8.2 cm square glass slides (Kodak Ltd.). The gel was left to solidify,

and allowed to stabilise for 1 - 2 hours at 4°C. Wells were then cut into the agarose using a standard circular immunodiffusion template consisting of a central well 7 mm diameter, and 8 peripheral wells each 3 mm diameter, the wells separated by a distance of 10 mm. Peripheral wells were filled with undiluted ANA positive patient sera, or ANA negative normal control sera, and the central well finally filled with a 1mg/ml solution of commercial calf-thymus 'DS' DNA (p. 76) or Nuclease SI 'purified' commercial calf-thymus 'DS' DNA (p. 77) in 0.01 M phosphate buffer. The plates were incubated horizontally in a moist chamber at 4°C for 42 hours, and were examined by transillumination at 18 hours and 42 hours for lines of immunoprecipitation. The results were recorded by photography.

Ouchterlony Double Diffusion Test for SS-DNA Precipitins

ANA positive sera were examined for precipitins to single-stranded DNA using the Ouchterlony double-diffusion test described above, but with calf-thymus single-stranded DNA, 1 mg/ml (p.79), as the antigen in the central well. In addition, care was taken to conduct all technical procedures at temperatures not exceeding 15°C to avoid single-strand recoil.

Controls to Exclude Non-immunological Precipitation with DNA

These controls were performed on a random selection of sera with positive Ouchterlony precipitins to commercial calf-thymus 'DS'-DNA:

1. Confirmation that precipitation was still observed when Ouchterlony tests were repeated using sera heat inactivated at 56°C for 30 minutes to attempt to exclude non-specific binding of DNA with Clq (Hughes, 1975).

2. Demonstration of immunoglobulin in Ouchterlony precipitin lines by autoradiography with I^{125}-labelled antihuman immunoglobulin (Plates 2 & 3), and evidence of its identity as IgG immunoglobulin using immunoelectrophoresis (Plate 4).

3. Failure to obtain Ouchterlony precipitin lines using sera absorbed with antihuman immunoglobulin (Plate 5).

4. Confirmation of Ouchterlony results using counter-current electroimmunoprecipitation (Johnson, Edmonds and Holborow, 1973), a method which separates immunologically specific from non-specific precipitation making use of the cathodal drift of gamma globulins (despite their net negative charge), which separates them from serum proteins such as Clq which remains at, or near, the anodal well (Plate 6). The method is the same as that described in detail for use with RNA (p.105).

Ouchterlony gel double-diffusion showing line of precipitation of a component in an SLE patient's serum (undiluted) diffusing from peripheral well 2 with calf thymus 'double-stranded' DNA test antigen (1 mg/ml) diffusing from the central well. (Magnification x 4)

Autoradiograph showing I^{125}-labelled rabbit antihuman immunoglobulin bound to the precipitin line shown above in Plate 2 indicating the presence of human immunoglobulin. (Magnification x 4)

Plate 2

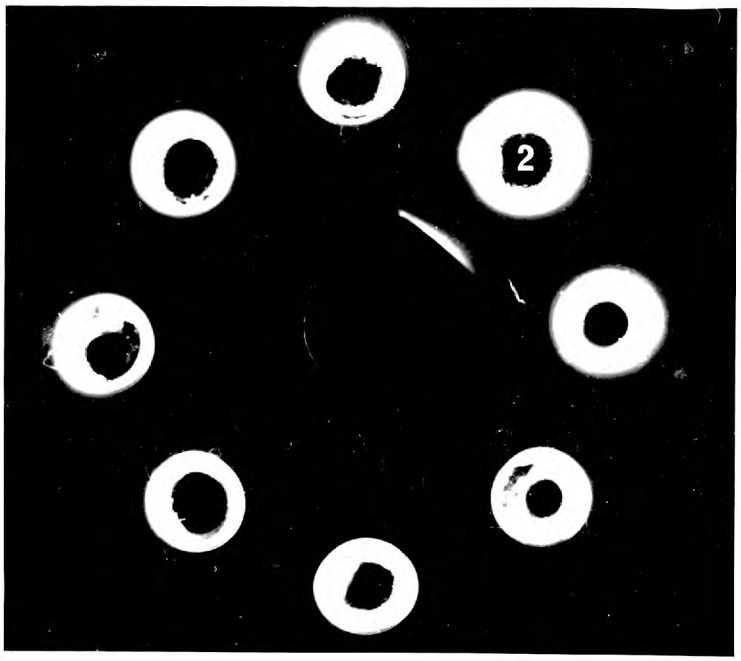
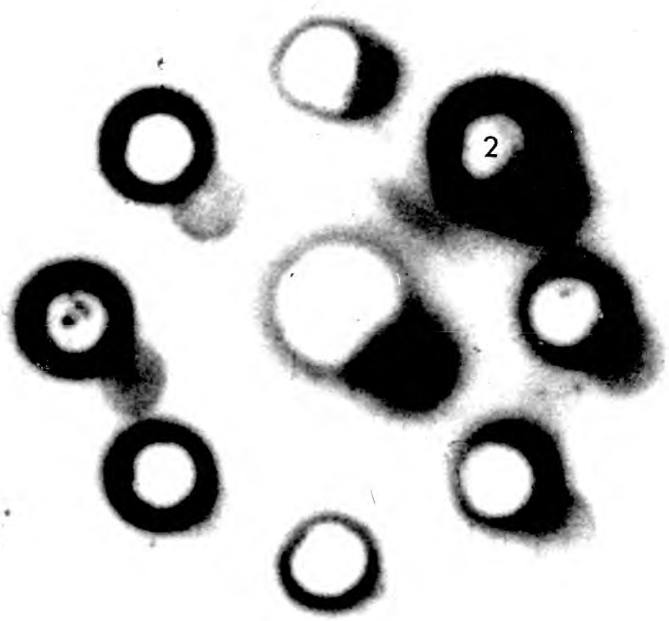


Plate 3



The characteristic immunoelectrophoretic precipitation pattern (1) (in duplicate) of an undiluted DNA precipitin positive serum in the top well against calf thymus 'DS'-DNA (1 mg/ml) in the top two troughs. Shown for comparison are immunoelectrophoretic precipitation patterns characteristic of human IgG (2) using DNA precipitin negative sera in the bottom two wells against goat anti-human IgG in the bottom trough. (Magnification x 2)

Ouchterlony gel double-diffusion showing positive precipitations of a patients serum from peripheral wells 1 and 3 with calf thymus 'DS'-DNA from the central well but absence of precipitation using the same serum pre-absorbed with rabbit antihuman immunoglobulin in wells 2 and 3. (Magnification x 4)

Plate 4

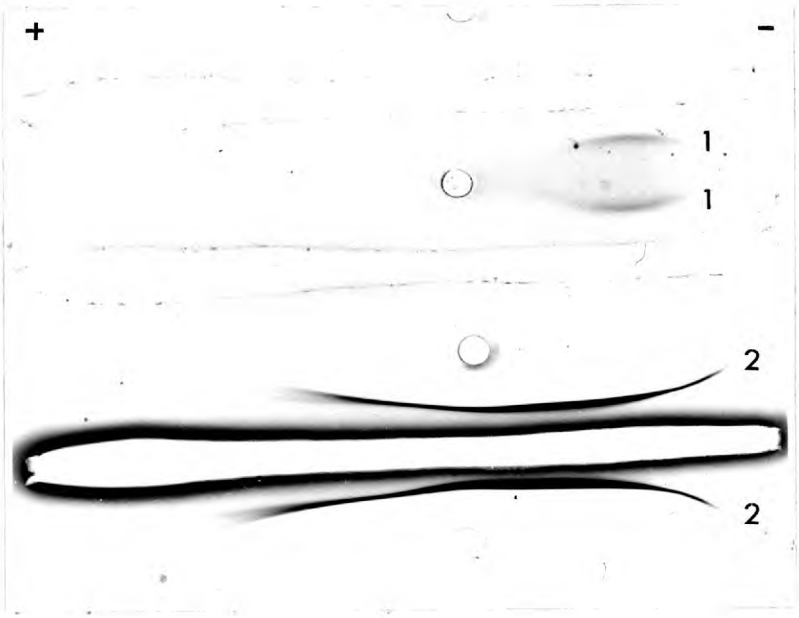
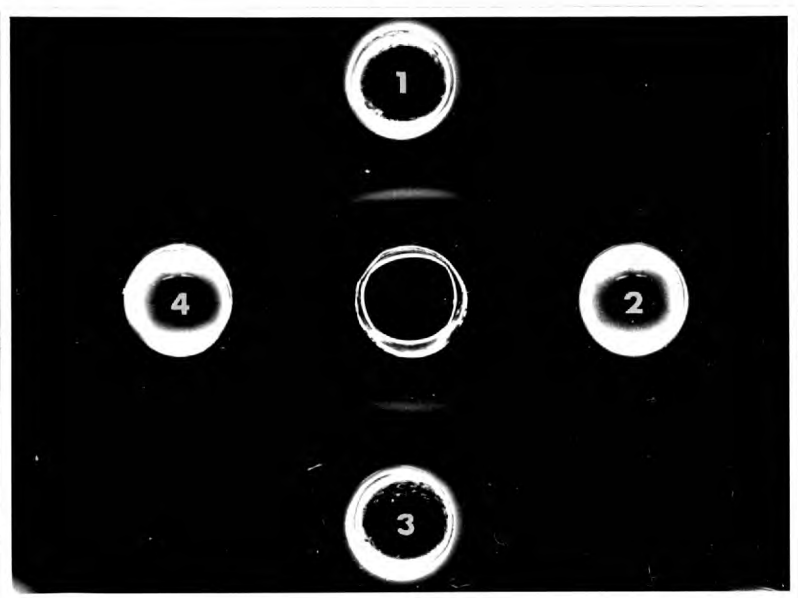
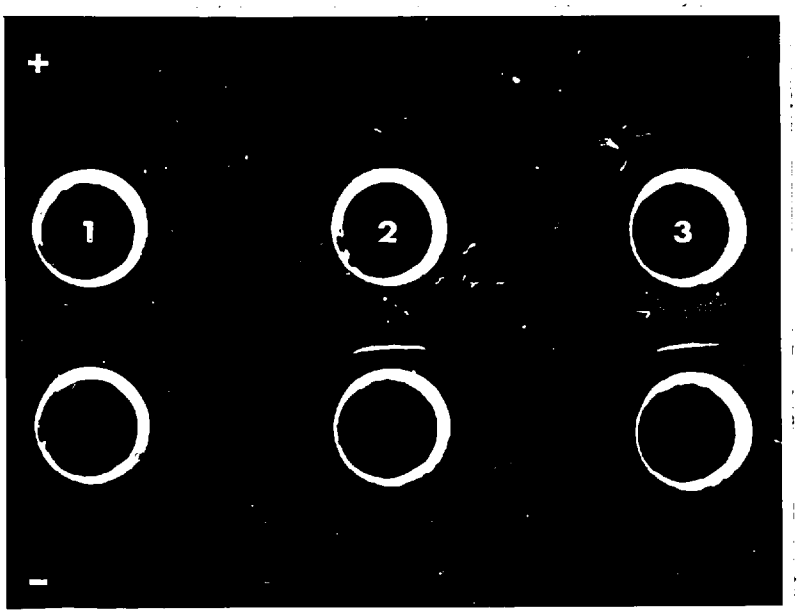


Plate 5



DNA precipitins demonstrated by counter-current (two stage) electroimmuno-precipitation: Undiluted sera from three SLE patients were applied to the anodal (+) wells in the first electrophoretic stage, and calf thymus 'DS'-DNA (20 µg/ml) to the cathodal (-) wells in the second stage. Sera 2 and 3 show lines of specific immunoprecipitation with DNA neighbouring the cathodal wells which are clearly distinct from hazy non-specific precipitation neighbouring the anodal wells. Serum 1 shows neither specific nor non-specific precipitation with DNA. (Magnification x 6)

Plate 6



Farr Assay for 'DS' DNA Binding Antibodies

(All the DNA binding assays included in the present study were kindly performed by Dr. Ian Griffiths of the Kennedy Institute of Rheumatology).

The binding capacity of serum globulins with DNA was measured using a Farr assay (Farr, 1958; Wold et al, 1968) modified by Holian et al (1975).

Test sera were heated at 56°C for 30 minutes to inactivate complement, then diluted to 10% serum concentration in 0.15M borate buffer (pH 7.8). 50 µl of this solution was reacted with 0.05 µg of I^{125}-labelled HeLa cell DNA (p.79) for 1 hour at 37°C, and a further 18 hours at 4°C.

An equal volume of saturated ammonium sulphate was then added, and the mixture immediately centrifuged at 1,500g for 15 minutes. The supernatant was aspirated and the radioactivity of the precipitate measured.

Results were expressed as units of binding activity in relation to known reference standards (Holian et al, 1975), the upper normal limit being 25 units/ml of activity.

METHODS TO DETECT ANTIBODIES TO RIBONUCLEATE ANTIGENS

These methods were used to examine a small number of ANA positive sera, particularly those with speckled and nucleolar immunofluorescent staining patterns.

Ouchterlony Double-diffusion Test for RNA Precipitins

The method used was essentially the same as that previously described to detect 'DS' and SS DNA precipitins but because the total yeast RNA antigen was insoluble in 0.01 M buffers the tests, including preparation of agarose and RNA solutions were conducted in three different buffers (p.80).

The antigen was depolymerized total yeast RNA comparing 5 mg/ml and 1 mg/ml solutions.

The total immunodiffusion period was extended to 5 days and plates examined daily.

Counter-current Electroimmunoprecipitation Test for RNA Precipitins

The modification of the test used was that of Johnson, Edmonds & Holborow (1973). Agarose was dissolved at 1.2% concentration in 0.05 M sodium barbitone/HCl buffer pH 8.2, and applied in 10 ml volumes to

8.2 x 8.2 cm square glass slides (Kodak Ltd.). After the gel had set and stabilized at 4°C for 1 - 2 hours, wells were cut using a standard template consisting of two rows of paired wells (anodal and cathodal) 3 mm diameter, centres 6mm apart, and with 7 pairs of wells per row. Two such templates were accommodated per plate.

Plates were positioned for electrophoresis using the barbitone buffer, a constant voltage of 110V and varying the resistance by adjusting the wick thickness to achieve a constant current of 12 m amps per slide, and a cooling plate was used to achieve a constant temperature of 10°C.

10 µl volumes of undiluted ANA positive sera or ANA negative control sera were applied to the anodal wells and electrophoresis continued for 45 minutes to achieve an effective cathodal gamma globulin drift. The power was momentarily disconnected and total yeast RNA antigen solutions (p.80) (200, 100 and 20 µg/ml) were applied to the cathodal wells. Electrophoresis was recommenced for a further 15 minutes to achieve migration of RNA towards the anodal wells. Plates were examined for lines of immediate immunoprecipitation and again after overnight (18 hours) incubation at 4°C, then photographed.

Immunofluorescent Test for Antibodies to RNAase Sensitive Antigens

The method used was that of Sharp et al, 1972. For each batch of tests 1 litre of 0.15M sodium chloride was prepared, the pH adjusted to 7.0 and magnesium chloride added to a final 0.4 mM concentration. Using this buffer, two enzyme solutions were prepared, one 300 ml containing 0.04 mg/ml ribonuclease A (p. 73), and the other 300 ml containing 0.04 mg/ml deoxyribonuclease 1 (p. 73).

Triplicate sets of microscope slides bearing air-dried cryostat cut sections of fresh rat liver, 2-4 μ thick, were placed in glass slide container dishes, and one set immersed in the RNAase solution to remove RNAase -sensitive tissue antigens, one set in the DNAase solution to remove DNAase sensitive tissue antigens, and the remaining set in the enzyme-free saline solution as controls.

The immersed sections were incubated at 37°C for 90 minutes, then washed thoroughly in 3 changes of fresh Coon's barbitone immunofluorescent staining buffer, pH 7.2 (p.85).

A small number of sera, including most of those with speckled or nucleolar immunofluorescent staining patterns, were retested on these pre-treated rat liver sections using the immunofluorescent staining test for ANA previously described (p. 85), and any change in staining patterns recorded.

V. METHODS TO STUDY LYMPHOCYTES

LEUCOCYTE MIGRATION TEST (LMT).

This was first described as an in vitro test to demonstrate cell-mediated hypersensitivity by Søborg and Bendixen, 1967.

The technique used was the micromethod of Federlin et al (1971), including most of the modifications advised by Maini et al (1973).

Leucocytes were, however, obtained from defibrinated blood since it was found, in agreement with Brogan (1972) and Foulis (1972), that formation of cell-associated fibrin in heparinised blood causes variable cell clumping in capillaries and poor test reproducibility. The method used was that of Falk, Collste & Möller (1970). Forty millilitres of venous blood was defibrinated in a siliconised 100 ml conical flask by swirling round a glass defibrination rod (Dacie and Lewis, 1963). The blood was divided between two plastic universals, 3% gelatin in physiological saline added to each (one part gelatin to three parts blood), and the mixture incubated for 20 to 30 minutes to sediment red cells (Coulson and Chalmers, 1964). The supernatants were centrifuged at 250g for ten minutes, and the leucocyte rich cell pellet further depleted of red cells by lysis with 0.83% NH_4Cl solution for 5 minutes using nine parts NH_4Cl to one part cell suspension (Strander and Cantell, 1966).

The leucocytes were washed twice in EBMS (p.81) and resuspended. Siliconized microcapillary tubes, ten microlitre volume, were filled

with cell suspension, plugged with Seal-Ease clay and spun at 250g for ten minutes having adjusted the concentration of cell suspension to give a 3mm packed cell length. With this method, 40 to 60 microcapillaries could be prepared from 40 ml of venous blood.

Capillary tubes were cut just below the cell/fluid interface and laid singly in individual wells of disposable polystyrene tissue culture chambers (Steralin Ltd.). Test chambers were filled with EBMS containing antigen, and control chambers with EBMS and sealed with cover slips. After incubation at 37°C for 18 hours, areas of migration were measured by projection and planimetry. The results were expressed as a percentage inhibition:

$$\% \text{ inhibition} = 100 - \frac{\text{Migration area in presence of antigen} \times 100}{\text{Migration area in absence of antigen}}$$

All tests were performed in triplicate, and reproducibility between triplicate migration areas was good with coefficients of variation not greater than 10% and often less than 5%.

TRYPAN BLUE VIABILITY TESTS

Viability of leucocytes before and after culture with antigen was confirmed by their ability to exclude trypan blue.

CONSTRUCTION OF DOSE RESPONSE CURVES

Dose response curves were constructed by testing with five or six different concentrations of each antigen ranging from 2000 to 50 µg/ml for 'DS'-DNA and whole nuclei, 250 to 50 µg/ml for 'SS'-DNA and RNA and 100 to 6.25 µg/ml for deoxyribonucleoprotein. From these curves optimum concentrations which combined minimum toxicity with maximum sensitivity were selected to compare patients and controls. For the results reported here antigen concentrations selected were 100 and 50 µg/ml for 'DS'-DNA, nuclei, 'SS'-DNA and RNA, and 12.5 and 6.25 µg/ml for deoxyribonucleoprotein.

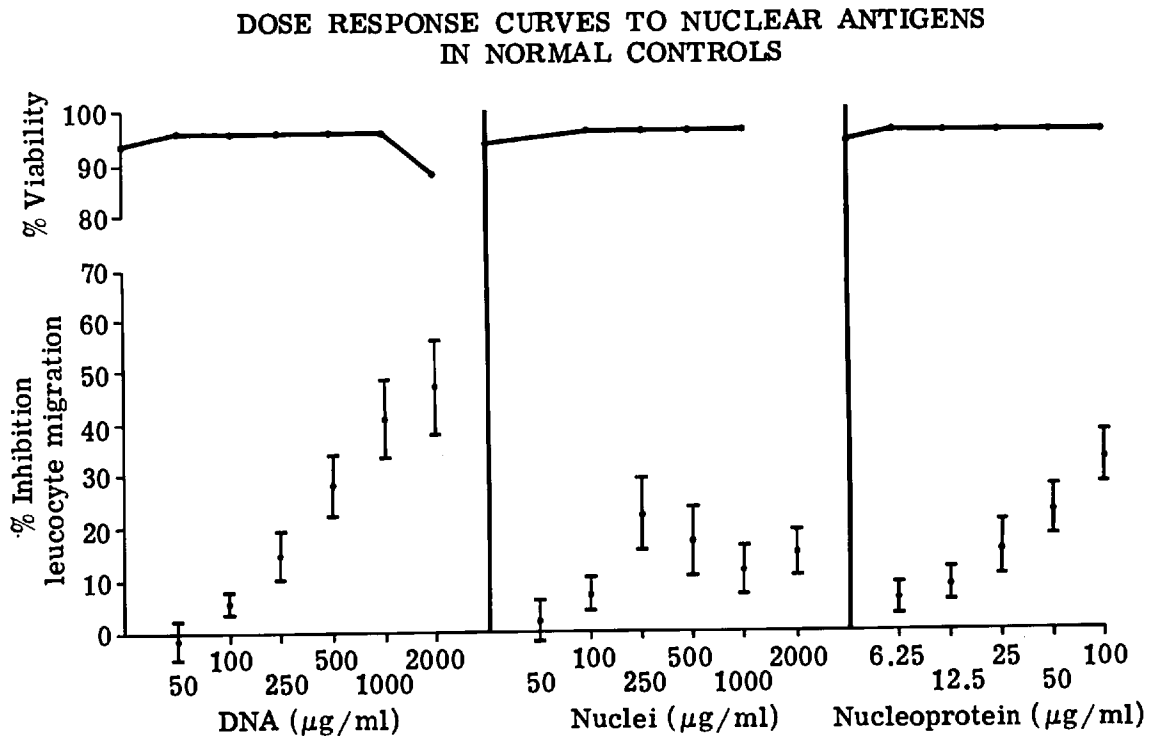
Figure 1. demonstrates typical combined dose response/viability studies used to select optimum test doses of antigens.

CONTROLS FOR LYMPHOKINE INDEPENDENT INHIBITION

To reasonably exclude some possible non-lymphokine mechanisms for inhibition of leucocyte migration before equating positive inhibition with specific T-lymphocyte sensitization, the following methods were used:

1. Inhibition of migration due to cell damage or agglutination by antigens was excluded by using adequate dose-response curves and checking cell viability with trypan blue.

Figure 1



I Mean inhibition and standard error of the mean of triplicate tests in sixteen normal healthy controls

2. Possible antibody-mediated inhibition due to cross-linking of cells producing antibody (Makela and Nossal, 1961) was examined in a few instances by looking for evidence of immunofluorescent ANA in supernatants from 6 day cultures of peripheral leucocytes in the presence of antigen.

3. Possible antibody-mediated inhibition due to antigen/antibody complexes (Moller, 1969) was examined in a few instances by purposefully adding ANA positive serum to control leucocytes in the presence of antigen (with and without complement).

4. General confirmation of the leucocyte migration test results was obtained from a small number of studies using indirect guinea pig macrophage migration tests (George and Vaughan, 1962) and lymphocyte transformation tests (Ling 1968) as other in vitro tests of cell-mediated hypersensitivity.

ANTINUCLEAR ANTIBODY TESTS FOR CORRELATIONS

Serum taken from patients and controls at the time of leucocyte sampling was examined for immunofluorescent ANA, and ANA titres were measured. In addition, sera were tested for antibodies to

deoxyribonucleoprotein (p. 94) and for precipitins to commercial calf-thymus 'DS'-DNA (p. 95) using the same antigens as those used for the leucocyte studies to enable comparison of antibody and lymphocyte findings.

CHAPTER 4

RESULTS AND CORRELATIONS

1. RESULTS OF STUDIES ON PREVALENCE OF IMMUNOFLUORESCENT ANA

PREVALENCE OF IMMUNOFLUORESCENT ANA IN NORMAL CONTROLS

The prevalence of immunofluorescent ANA of titre 1 in 10 or greater (using polyvalent antihuman immunoglobulin) in serum from 205 normal control persons without lung disease (mean age 43, SD 16 years) was very low. Using the standardized immunofluorescent indirect staining technique, screening sera at a 1 in 10 dilution and disregarding suspect $\pm$ weak positive reactions, ANA was observed in 10 (5%) of the 205 normal control sera.

The Influence of Sex

The prevalence of immunofluorescent ANA in male and female normal controls of comparable age range was similar. ANA was observed in 5 (4%) out of 121 male normal controls (mean age 39, SD 15 years), and in 5 (6%) out of 84 female normal controls (mean age 44, SD 16 years).

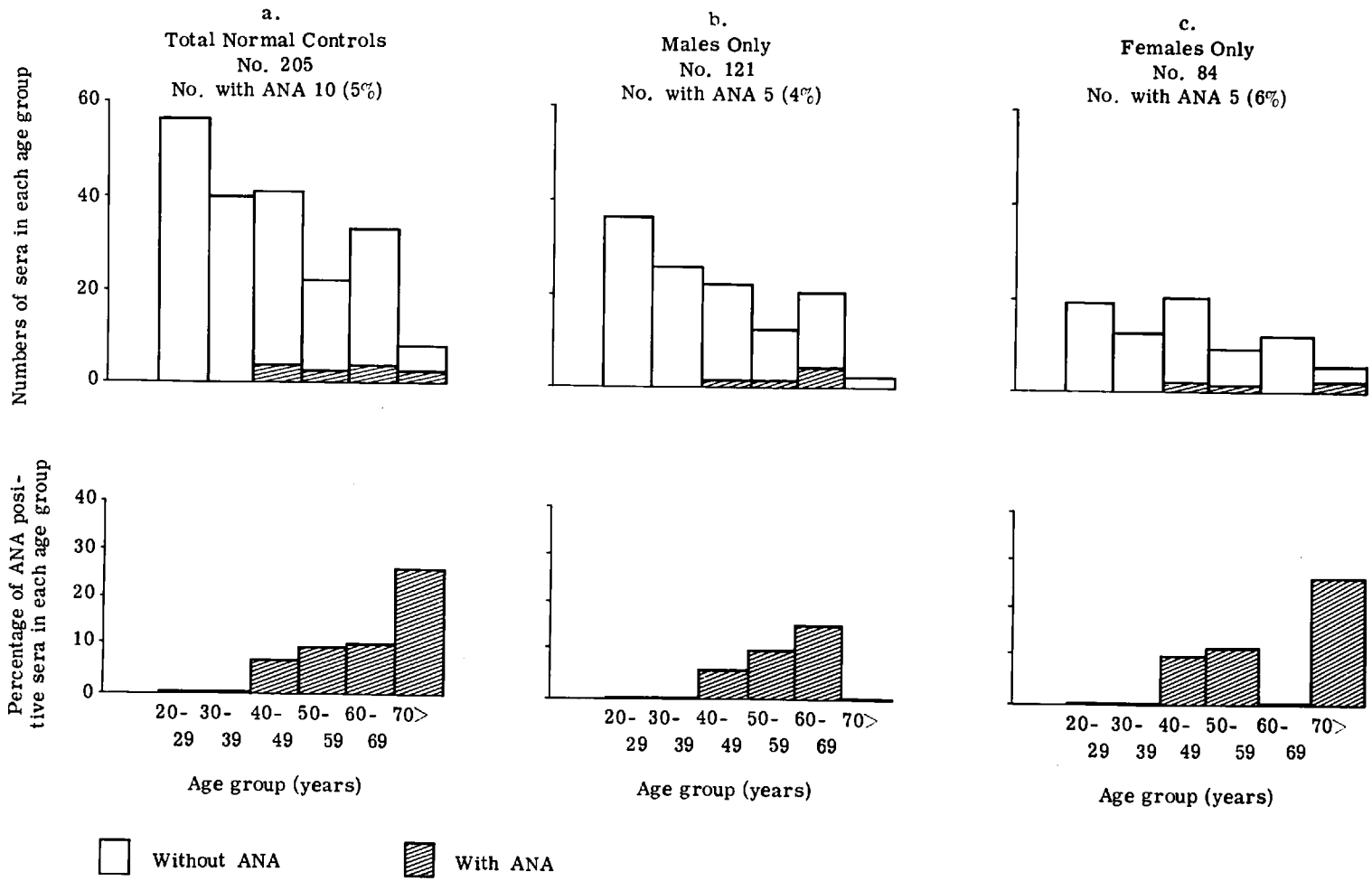
The Influence of Age

It was confirmed that the prevalence of immunofluorescent ANA increases with increasing age in a normal population (Figure 2).

This trend was demonstrated by subdividing the normal control population according to age. Juveniles of 0 to 19 years were excluded as nearly all patients in the present study were over 19 years old. The main age groupings chosen were five decades of 20 to

Figure 2.

Figure demonstrating that the prevalence of immunofluorescent ANA (titre $\geqslant$ 1 in 10) increases with increasing age in normal controls.



29; 30 to 39; 40 to 49; 50 to 59 and 60 to 69 years. In addition there was a less satisfactory group of 70 years and over, since it was difficult to obtain sufficient numbers of normal persons of this age.

Figure 2a shows the results for the total normal controls. The most significant finding was the complete absence of ANA in the younger 20 to 29, and 30 to 39 age groups (0 out of 58, and 0 out of 40 cases respectively), in contrast with an increased prevalence in the older age groups 7% (3 out of 43 cases) in the 40 to 49 age group, 9% (2 out of 23 cases) in the 50 to 59 age group, 9% (3 out of 33 cases) in the 60 to 69 age group and 25% (2 out of 8 cases) in the 70 and over age group.

These increases in the prevalence of ANA in the older (40 and over) age groups compared with the younger (under 40) age groups were all statistically significant, and the detailed probability levels for each difference are shown in Statistical Table 1a, Appendix.

The tendency for the prevalence of ANA to increase progressively with each decade after 40 years of age could not be confirmed by the statistical tests.

In summary the prevalence of ANA in normal controls of 40 years and over was 9% (10 out of 107 cases), significantly greater than in normal controls of under 40 years where the prevalence was 0% (0 out of 98 cases); $p < 0.01$.

Figure 2b and c, and Statistical Table 1 b and c (Appendix) show the results for males and females separately. The increase in the prevalence of ANA with increasing age was seen in both male and female normal controls but the trends were less clear than in the group as a whole in view of the smaller numbers. The complete results can be seen by reference to Figure 2b and c, and Statistical Table 1 b and c.

In summary, the prevalence of ANA in male normal controls of 40 years and over was 9% (5 out of 58 cases), significantly greater than in male normal controls of under 40 years where the prevalence was 0% (0 out of 63); $p < 0.02$.

Similarly, the prevalence of ANA in female normal controls of 40 years and over was 10% (5 out of 49 cases), significantly greater than in female normal controls of under 40 years where the prevalence was 0% (0 out of 35 cases); $p = 0.05$.

PREVALENCE OF IMMUNOFLUORESCENT ANA IN LUNG DISEASE IN COMPARISON WITH NORMAL CONTROLS

Table 4 shows the prevalence of immunofluorescent ANA of titre 1 in 10 or greater (using polyvalent antihuman immunoglobulin) in patients with various lung diseases in comparison with the prevalence

TABLE 4

A comparison of the prevalence of immunofluorescent ANA (titre ≥ 1 in 10) in patients with different lung diseases, and in normal controls of comparable age. (Statistical comparisons using a test of difference of proportions).

ANA prevalence in patients with different lung diseases				ANA prevalence in normal controls of comparable age			Significant increase over controls
Disease	No. of patients	No. with ANA	% with ANA	No. of controls	No. with ANA	% with ANA	p ≤
Pleuropulmonary SLE	28	28	100	205	10	5	0.001
CFA	142	53	37	147	10	7	0.001
Asbestosis	74	19	26	99	8	8	0.01
Asbestos exposure	74	3	4	99	8	8	NS
Sandblaster's silicosis	30	13	43	106	5	5	0.001
Coal miner's pneumoconiosis	109	19	17	107	10	9	0.1
Acute EAA	20	3	15	197	8	4	0.05
Chronic EAA	34	1	3	99	8	8	NS
Chronic sarcoidosis	33	3	9	197	8	4	NS
Chronic bronchitis	37	6	16	107	10	9	NS
Intrinsic asthma	48	13	27	197	8	4	0.001
Extrinsic asthma	43	6	14	164	5	3	0.01

in normal controls of comparable mean age and age range (within 5 years) from the main normal control population who were studied using exactly the same method. The age range in all the disease groups extended beyond 40 years (p. 58) where an age associated increase in the prevalence of ANA was demonstrated in normal controls. (Immunofluorescent ANA of titres $\geq$ 1 in 10 was a selective diagnostic criterion for pleuropulmonary SLE but the group has been included in the Tables and text for comparative purposes).

Diseases where the prevalence of ANA was confirmed to be significantly above that in normal controls of comparable age were, at the highest levels of significance, cryptogenic fibrosing alveolitis, with ANA in 37% (53 out of 142 cases), $p < 0.001$; sandblasters' silicosis in 43% (13 out of 30 cases), $p < 0.001$ and intrinsic asthma in 27% (13 out of 48 cases), $p < 0.001$. (The prevalence of ANA in normal controls of comparable age to the patients with pleuropulmonary SLE was only 5%; $p < 0.001$). Still significant but at lower levels were asbestosis in 26% (19 out of 74 cases), $p < 0.01$; extrinsic asthma in 14% (6 out of 43 cases), $p < 0.01$; acute extrinsic allergic alveolitis in 15% (3 out of 20 cases), $p < 0.05$; and coal miners' pneumoconiosis in 17% (19 out of 109 cases), $p < 0.1$.

Diseases where the prevalence of ANA was not significantly above that in normal controls of comparable age were chronic extrinsic allergic alveolitis with ANA in 3% (1 out of 34 cases), chronic sarcoidosis in 9% (3 out of 33 cases); and chronic bronchitis in 16% (6 out of 37 cases).

A special additional control group was included for comparison with the asbestosis disease group, consisting of workers matched for age, sex and duration of dust exposure with the disease group but without lung disease, to determine whether dust irritation alone can enhance ANA production. The prevalence of ANA was not significantly increased in this special control group.

Lung diseases with an increased prevalence of ANA were also found to differ significantly from each other with regard to the proportion of cases with ANA. The differences are shown in Statistical Table 2, Appendix. The proportion of cases with ANA in cryptogenic fibrosing alveolitis (37% ANA positive cases) and sandblasters' silicosis (43% ANA positive cases) were similar, both had significantly fewer cases with ANA than pleuropulmonary SLE, but had a greater proportion of cases with ANA than any of the remaining lung diseases. In the remaining diseases, although the prevalence of ANA varied from 26% in asbestos is to 14% in extrinsic asthma, these differences were not significant in the statistical analysis.

The Influence of Sex

The results are shown in Tables 5 and 6. (Detailed information with regard to sex was not available for the intrinsic asthma group).

Differences in the prevalence of ANA in the different lung diseases were not a reflection of sex differences. Table 5 shows the prevalence of ANA in male patients from each disease group in comparison with male normal controls. The results were similar to those for the

TABLE 5

A comparison of the prevalence of immunofluorescent ANA (titre ⩾ 1 in 10) in male patients with different lung diseases, and in male normal controls of comparable age. (Statistical comparisons using a test of difference of proportions).

ANA prevalence in patients with different lung diseases				ANA prevalence in normal controls of comparable age			Significant increase over controls
Disease	No. of patients	No. with ANA	% with ANA	No. of controls	No. with ANA	% with ANA	P ⩽
Pleuropulmonary SLE	7	7	100	56	5	9	0.001
CFA	86	27	31	58	5	9	0.01
Asbestosis	66	18	27	56	5	9	0.01
Asbestos exposure	72	3	4	56	5	9	NS
Sandblaster's silicosis	29	12	41	106	5	5	0.001
Coal miner's pneumoconiosis	109	19	17	68	5	7	0.1
Acute EAA	12	2	17	85	1	1	0.01
Chronic EAA	24	0	0	56	5	9	NS
Chronic sarcoidosis	15	2	13	119	5	4	NS
Chronic bronchitis	27	5	19	36	4	11	NS
Extrinsic asthma	18	2	11	98	2	2	0.1

TABLE 6

A comparison of the prevalence of immunofluorescent ANA (titre ⩾1 in 10) in female patients with different lung diseases, and in female normal controls of comparable age. (Statistical comparisons using a test of difference of proportions).

ANA prevalence in females with different lung diseases				ANA prevalence in female normal controls of comparable age			Significant increase over controls
Disease	No. of patients	No. with ANA	% with ANA	No. of controls	No. with ANA	% with ANA	P ⩽
Pleuropulmonary SLE	21	21	100	84	5	6	0.001
CFA	56	26	46	63	5	8	0.001
Asbestosis	8	1	13	22	1	5	NS
Asbestos exposure	2	0	0	22	1	5	NS
(Sandblaster's silicosis	1	1	100	21	2	10	NS)
Acute EAA	8	1	13	63	5	8	NS
Chronic EAA	10	1	10	43	3	7	NS
Chronic sarcoidosis	18	1	6	66	3	5	NS
Chronic bronchitis	10	1	10	84	5	6	NS
Extrinsic asthma	25	4	16	78	3	4	0.05

total patients, confirming significant increases in the prevalence of ANA in cryptogenic fibrosing alveolitis 31% (27 out of 86 males), asbestosis 27% (18 out of 66 males), sandblasters' silicosis 41% (12 out of 29 males), coal miners' pneumoconiosis 17% (19 out of 109 males), acute extrinsic allergic alveolitis 17% (2 out of 12 males) and extrinsic asthma 11% (2 out of 18 males).

Table 6 shows the prevalence of ANA in female patients from each disease group in comparison with female normal controls. Coal miners' pneumoconiosis has been omitted since this was a male group.
The results showed significant increases in the prevalence of ANA in cryptogenic fibrosing alveolitis 46% (26 out of 56 females) and extrinsic asthma 16% (4 out of 25 females).

Of these diseases with reasonable numbers of both male and female patients, cryptogenic fibrosing alveolitis was the only disease where the prevalence of ANA was difference in males and females. It was significantly higher in females 46% (26 out of 56 females) than in males 31% (27 out of 86 males); $p < 0.1$.

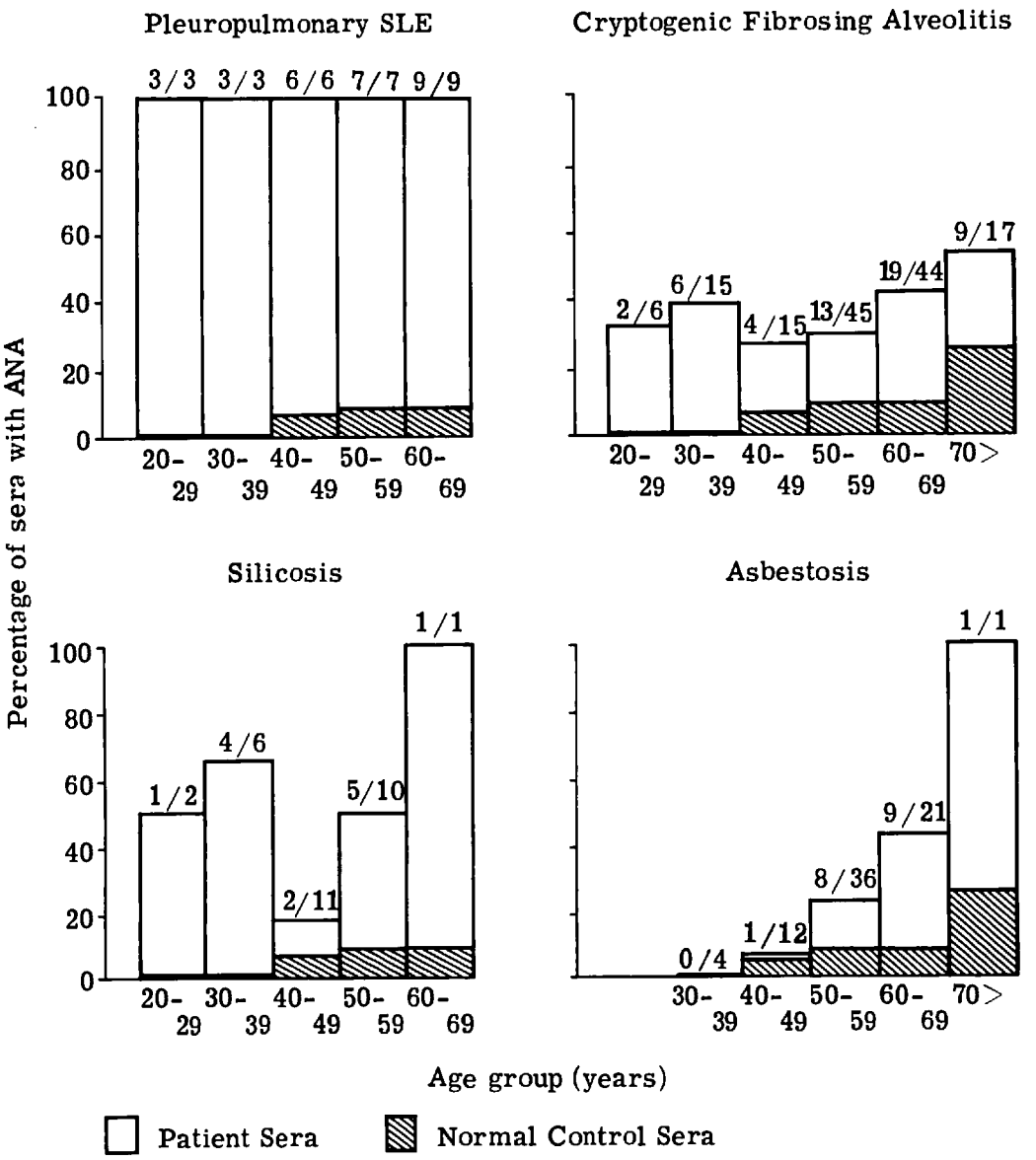
The Influence of Age

Diseases with an increased prevalence of ANA where there were sufficient numbers to investigate the influence of age were pleuropulmonary SLE, cryptogenic fibrosing alveolitis, asbestosis and sandblasters' silicosis. The results are shown in Figure 3 and Statistical Table 3, Appendix.

The influence of age was examined by subdividing each disease population according to age into groups of 20 to 29; 30 to 39; 40 to 49; 50 to 59; 60 to 69 and 70

Figure 3.

Figure demonstrating that the increases in prevalence of immunofluorescent ANA (titre$\geqslant$ 1 in 10) in different lung diseases are not age-related except in asbestosis.



NB. The number of the total sera with ANA in each age group is indicated at the head of each column.

years and over; and making comparisons of the ANA prevalence in these different age groups (Figure 3 and Statistical Table 3b). In addition, by making comparisons with the prevalence in normal controls of the same age groups (Figure 3 and Statistical Table 3a).

The increases appeared to be independent of age in all but asbestosis. In pleuropulmonary SLE, cryptogenic fibrosing alveolitis and sand-blasters' silicosis, increases in the prevalence of ANA above normal were apparent in younger as well as older age groups, and there were no significant differences between the age groups. However, in asbestosis (where the majority of the patients were in the age groups 40 to 49, 50 to 59 and 60 to 69 years) a trend for the prevalence of ANA to increase with age was clearly seen from a low 8% prevalence (1 out of 12 cases) in the 40 to 49 age group, to 22% (8 out of 36 cases) in the 50 to 59 age group increasing finally to 43% (9 out of 21 cases) in the 60 to 69 age group. The increase was significantly above that in normal controls of the same age only in the oldest 60 to 69 age group; $p < 0.01$; and the increase in prevalence from 8% in the 40 to 49 age group to 43% in the 60 to 69 age group was also significant; $p < 0.05$.

SELECTION OF LUNG DISEASE GROUPS FOR FURTHER STUDY

As a result of this preliminary investigation, immunofluorescent ANA

positive cases from those lung diseases where the increased prevalence of ANA was confirmed with a high degree of confidence were selected for more detailed studies. The diseases were pleuro-pulmonary SLE, cryptogenic fibrosing alveolitis, asbestosis, sand-blasters' silicosis and intrinsic asthma.

The investigations from this point onwards have been concerned only with ANA positive cases (p .61).

11. TITRES OF IMMUNOFLUORESCENT ANA (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN)

This was the first of a series of special investigations on the same immunofluorescent ANA positive serum samples to enable satisfactory correlations to be made.

NORMAL CONTROL STUDIES

The ANA titres in the ten ANA positive sera from normal controls without lung disease were low, never exceeding 1 in 40. Seven had the minimum positive titre of 1 in 10, two had titres of 1 in 20, and one had a titre of 1 in 40.

LUNG DISEASE STUDIES

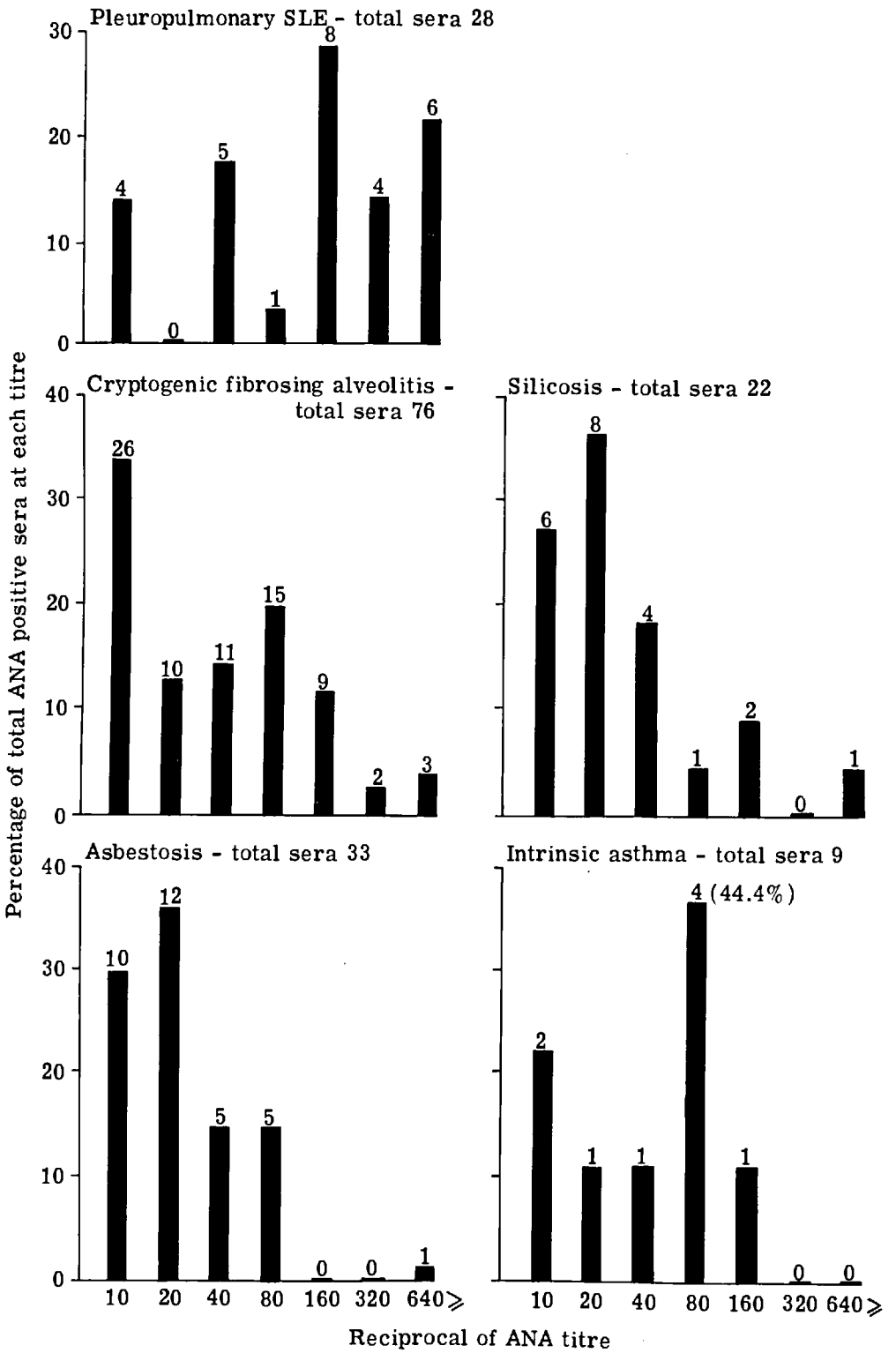
The sera used in this investigation were ANA positive sera from twenty eight patients with pleuropulmonary SLE; seventy six patients with cryptogenic fibrosing alveolitis; thirty three patients with asbestosis; twenty two patients with silicosis and nine patients with intrinsic asthma.

Figure 4 shows that a range of different ANA titres was present in each lung disease group. Pleuropulmonary SLE showed a distribution of ANA titres which included a greater proportion of sera with higher ANA titres of 1 in 160, 1 in 320 and 1 in 640 than the other lung diseases, where the distributions tended towards the lower ANA titres of 1 in 80 or less. In pleuropulmonary SLE, 64% (18 out

Figure 4.

A comparison of the distribution of positive titres of immunofluorescent ANA ($\geqslant$ 1 in 10) in the different lung diseases.

(Actual numbers of sera at each titre are indicated at the head of each column)



of 28) of the total ANA positive sera had ANA titres of 1 in 160 or greater. In contrast, there were only 19% (14 out of 76) in cryptogenic fibrosing alveolitis; 13% (3 out of 22) in silicosis; 11% (1 out of 9) in intrinsic asthma and 3% (1 out of 33) in asbestosis.

A more exact comparison of ANA titre distributions was made by determining the mean ANA titre for each disease group and the extent of variability around the mean (the standard deviation (SD) of the mean). The results are shown in Table 7 with detailed statistical comparisons in Statistical Table 4, Appendix.

Logarithms were used to determine the mean ANA titre and the SD for each group of ANA positive sera to satisfy the requirement for the statistical test used that groups compared should show a normal distribution.

The highest mean ANA titre was in pleuropulmonary SLE, mean 2.08 log. reciprocal titre (between 1 in 80 and 1 in 160); followed by intrinsic asthma, mean 1.63 log. reciprocal titre (approximately 1 in 40); then cryptogenic fibrosing alveolitis, mean 1.56 log. reciprocal titre (between 1 in 20 and 1 in 40); silicosis, mean 1.45 log. reciprocal titre (between 1 in 20 and 1 in 40); and lowest in asbestosis, mean 1.39 log. reciprocal titre (approximately 1 in 20).

TABLE 7

A comparison of the immunofluorescent ANA titre distributions in positive sera in the different lung diseases (1 in 10 the minimum titre accepted as positive).

Disease	Total No. of ANA pos. sera	Number and % of total sera at each titre														$\dagger$Mean ANA titre	S.D.
		Reciprocal of ANA titre															
		10		20		40		80		160		320		640			
		Logarithm of reciprocal of ANA titre															
		1.0		1.3		1.6		1.9		2.2		2.5		2.8			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
SLE	28	4	14	0	0	5	18	1	4	8	29	4	14	6	21	2.08*	0.60
CFA	76	26	34	10	13	11	14	15	20	9	12	2	3	3	4	1.56	0.52
ASB	33	10	30	12	36	5	15	5	15	0	0	0	0	1	3	1.39**	0.40
SIL	22	6	27	8	36	4	18	1	5	2	9	0	0	1	5	1.45	0.47
AST	9	2	22	1	11	1	11	4	44	1	11	0	0	0	0	1.63	0.44

* Significantly higher range of titres than all other lung diseases.
** Significantly lower range of titres than in CFA.

(For the detailed statistical comparisons please refer to Appendix , Stat. Table 4).

N.B. % to nearest whole number.

$\dagger$Log. reciprocal.

The tendency for ANA titres to be higher in pleuropulmonary SLE than in any other lung disease was confirmed to be highly significant in the statistical tests, $p < 0.001$. Intrinsic asthma was, however, excluded from the statistical tests in view of the small number of sera. In addition, the tests showed that ANA titres in cryptogenic fibrosing alveolitis were slightly higher than in asbestosis, $p < 0.1$. It could not be confirmed that titres in cryptogenic fibrosing alveolitis were higher than in silicosis, nor that those in silicosis were higher than in asbestosis.

The Influence of Sex

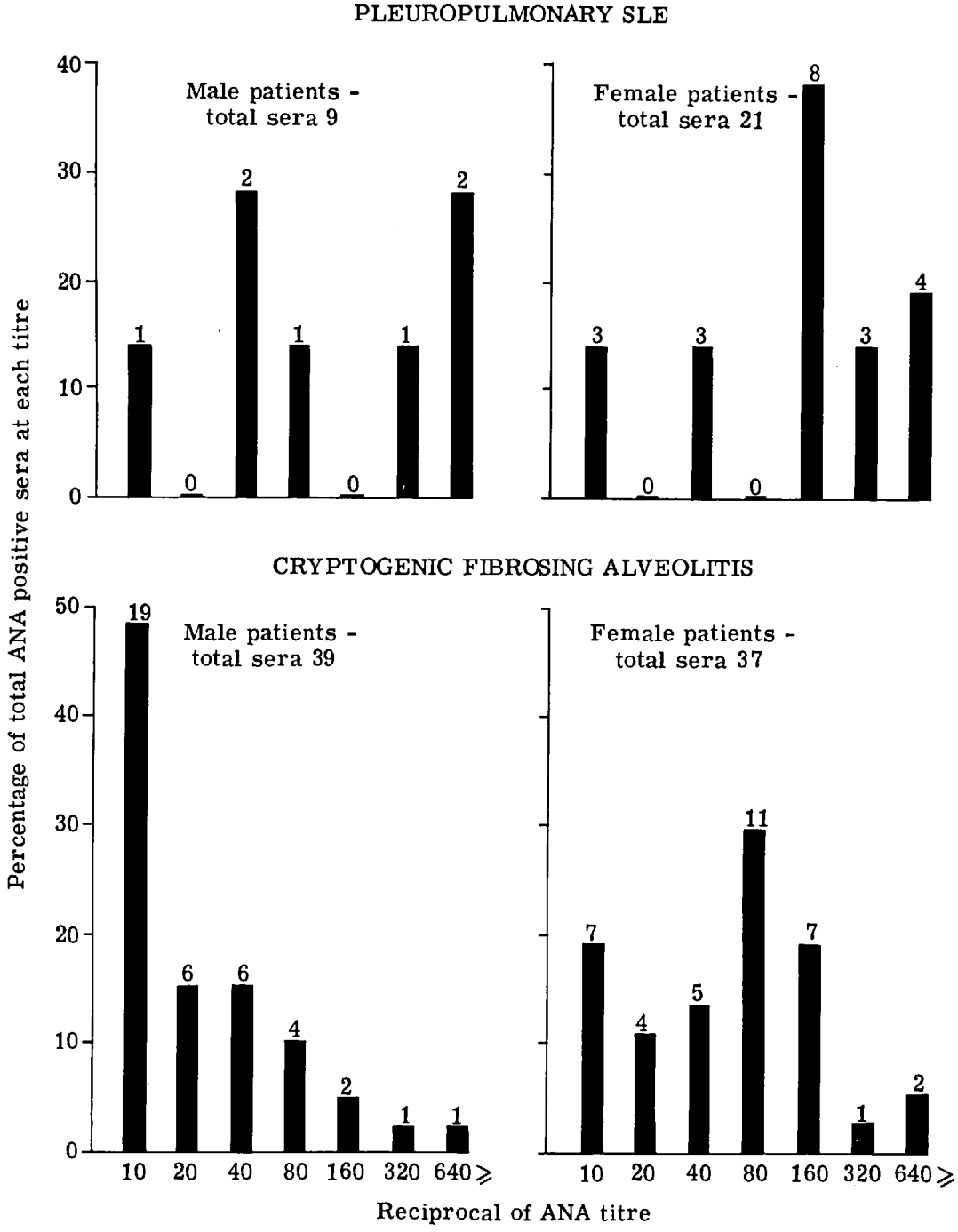
There was a relationship between the ANA titre and sex with a tendency for males to have lower titres of ANA than females, except in pleuropulmonary SLE, where ANA titres in males and females were similarly high.

Figure 5 shows the range of ANA titres separately for males and females in pleuropulmonary SLE and cryptogenic fibrosing alveolitis, which were the only groups with reasonable numbers of both males and females. In pleuropulmonary SLE the distribution of ANA titres in males and females were similar, involving both lower and higher ANA titres. In cryptogenic fibrosing alveolitis the distributions showed an obvious tendency towards higher ANA titres in females and lower ANA titres in the males.

Figure 5.

A comparison of the distributions of positive titres of immunofluorescent ANA (⩾1 in 10) in patients of different sex.

(Actual numbers of sera at each titre are indicated at the head of each column)



The more exact comparisons of the titres of immunofluorescent ANA in males and females from the five lung disease groups are shown in Table 8. In pleuropulmonary SLE it was confirmed that ANA titres in males, mean 2.03 log. reciprocal titre, were not significantly different from those in females, mean 2.10 log. reciprocal titre (between 1 in 80 and 1 in 160). In cryptogenic fibrosing alveolitis it was confirmed that ANA titres in females, mean 1.75 log. reciprocal titre (between 1 in 40 and 1 in 80), were significantly higher than in males, mean 1.38 log. reciprocal (approximately 1 in 20); $p < 0.01$ (Table 8). However, females with pleuropulmonary SLE still had significantly higher ANA titres than females with cryptogenic fibrosing alveolitis; $p < 0.05$ (Statistical Table 5a, Appendix).

It was also noted that males with pleuropulmonary SLE had significantly higher titres of ANA than males in any other disease group (Statistical Table 5b, Appendix) who had comparably low ANA titres with means of between 1 in 20 and 1 in 40.

The Influence of Age

Figure 6 shows the ANA titre for each positive patient correlated with the age of that patient in a conventional scatter diagram. The different diseases have not been distinguished. (Information with regard to age was missing in a few instances).

There was a complete absence of any correlation between ANA titre and age. (Correlation coefficient $r = -0.07$, not significant).

TABLE 8

A comparison of the immunofluorescent ANA titre distributions (≥ 1 in 10) in patients of different sex.

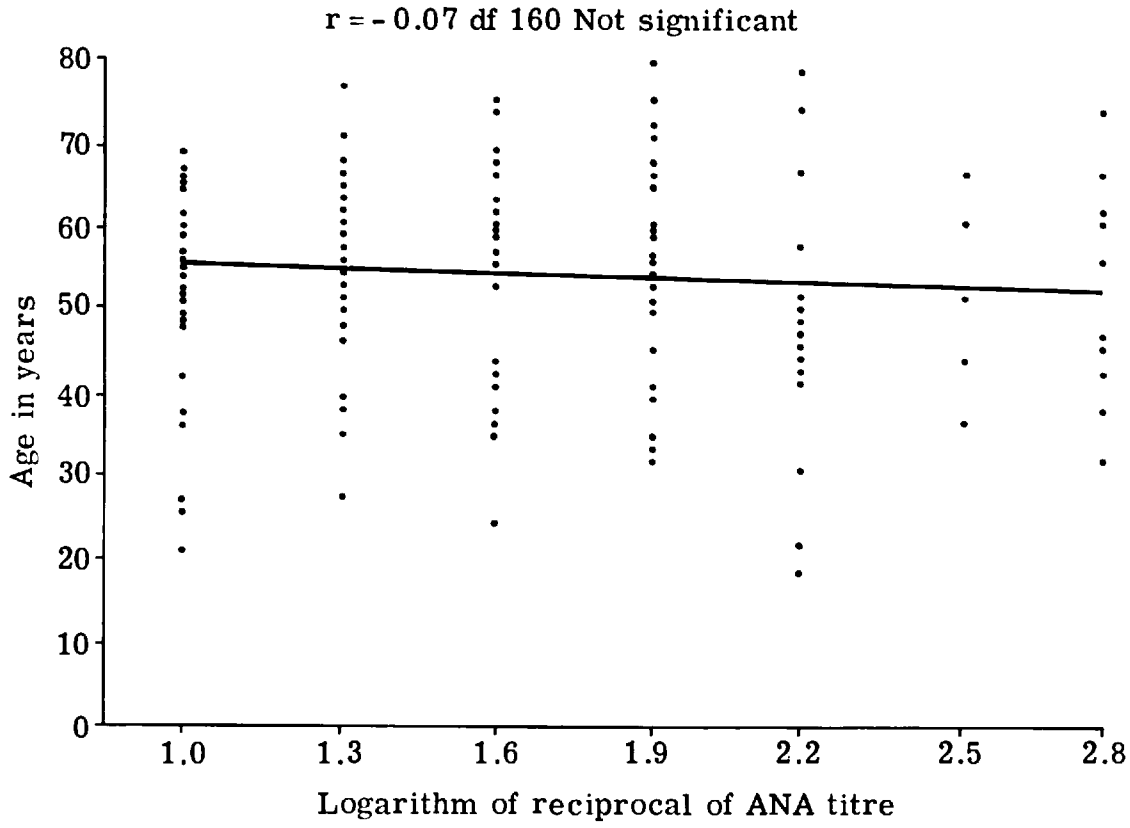
Disease	Sex	Total No. of ANA pos. sera	Number and % of total sera at each titre												Mean ANA titre†	S.D.	Sig. diff. P ≤*
			Reciprocal of ANA titre														
			10		20		40		80		160		320		640		
			Logarithm of reciprocal of ANA titre														
			1.0		1.3		1.6		1.9		2.2		2.5		2.8		
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	M	7	1	14	0	0	2	29	1	14	0	0	1	14	2	29	2.03	0.69	NS
	F	21	3	14	0	0	3	14	0	0	8	38	3	14	4	19	2.10	0.59	
CFA	M	39	19	49	6	15	6	15	4	10	2	5	1	3	1	3	1.38	0.48	0.01
	F	37	7	19	4	11	5	14	11	30	7	19	1	3	2	5	1.75	0.51	
ASB	M	30	8	27	12	40	5	17	4	13	0	0	0	0	1	3	1.40	0.39	NS
	F	3	2	67	0	0	0	0	1	33	0	0	0	0	0	0	1.30	0.51	
SIL	M	21	6	29	8	38	3	14	1	5	2	10	0	0	1	5	1.44	0.48	
	F	1	0	0	0	0	1	100	0	0	0	0	0	0	0	0	1.60	0	
AST	M	5	1	20	1	20	1	20	2	40	0	0	0	0	0	0	1.54	0.39	NS
	F	4	1	25	0	0	0	0	2	50	1	25	0	0	0	0	1.75	0.52	

*Statistical comparisons using an unpaired Student t test.
†Log. reciprocal.

N.B. % to nearest whole number.

Figure 6.

Absence of correlation between ANA titre and age demonstrated in a scatter diagram, and confirmed by calculation of the sample correlation coefficient r.



III. IMMUNOGLOBULIN CLASSES OF IMMUNOFLUORESCENT ANA

Sera were tested for IgG, IgM and IgA ANA, but not for IgE and IgD ANA.

NORMAL CONTROL STUDIES

The ten normal control sera (5 male and 5 female) positive in the standard immunofluorescent indirect staining test for ANA using polyvalent antihuman immunoglobulin, were retested with monovalent antihuman IgG, IgM and IgA.

The majority, 9 out of 10, had lone IgM ANA, and one (a female) had a combination of IgM with IgG ANA. There were no sera with IgA ANA.

LUNG DISEASE STUDIES

The ANA positive used for this investigation were the same as those used to investigate ANA titres. The numbers were, however, slightly fewer due to insufficient serum in some instances.

There were ANA positive sera from twenty seven patients with pleuropulmonary SLE; thirty patients with cryptogenic fibrosing alveolitis; twenty one patients with asbestosis; nineteen patients with silicosis and eight patients with intrinsic asthma.

Two different situations were seen; while a large number of sera contained only one immunoglobulin class of ANA, in contrast many other sera had a combination of two, or even three immunoglobulin classes of ANA.

For this reason the results have been considered in two ways; first (A) as the total sera with IgG, IgM and IgA ANA in each lung disease group; and secondly (B) as the profile of immunoglobulin classes of ANA in individual sera in each lung disease group.

A. The Total Sera with IgG, IgM and IgA ANA in the Different Lung Diseases

The numbers of immunofluorescent ANA positive sera with IgG, IgM and IgA ANA in each lung disease group are shown in Table 9 with statistical comparisons in Statistical Table 6, Appendix.

Antinuclear antibodies of all three immunoglobulin classes were present in every lung disease, but while IgG and IgM ANA's were common, IgA ANA's were relatively rare.

IgG ANA: The highest proportions of ANA positive sera with IgG ANA (Table 9) were in intrinsic asthma in 100% (8 out of 8) sera, pleuropulmonary SLE in 96% (25 out of 27) sera, and in silicosis

TABLE 9

The proportions of the total immunofluorescent ANA positive sera containing IgG, IgM and IgA ANAs.

Disease	Total no. of sera positive using polyvalent antihuman Ig	Number positive with antihuman IgG		IgM		IgA	
		No.	%	No.	%	No.	%
Pleuropulmonary SLE	27	26	96	20	74	9	33
CFA	30	23	77	21	70	9	30
Asbestosis	21	13	62*	15	71	3	14
Silicosis	19	17	89	7	37**	3	16
Intrinsic asthma	8	8	100	5	63	1	13
Total patients	105	87	83	68	65	25	24

*Significantly lower proportion of IgG ANA than all other diseases except CFA.
**Significantly lower proportion of IgM ANA than all other diseases except intrinsic asthma.
(For the detailed statistical analysis please refer to Appendix , Stat. Table 6).

N.B. % to nearest whole number.

in 89% (17 out of 19). The proportion was slightly lower in cryptogenic fibrosing alveolitis in 77% (23 out of 30); and lower still in asbestosis in 62% (13 out of 21) sera.

The statistical analysis (Statistical Table 6a) emphasized that asbestosis had significantly less IgG ANA than all other diseases, except cryptogenic fibrosing alveolitis. However, the slightly lower proportion of IgG ANA in cryptogenic fibrosing alveolitis was not significant except in comparison with pleuropulmonary SLE; $p < 0.05$.

IgM ANA: The highest proportions of ANA positive sera with IgM ANA (Table 9) were in pleuropulmonary SLE in 74% (20 out of 27) sera; asbestosis in 71% (15 out of 21%) sera; cryptogenic fibrosing alveolitis in 70% (21 out of 30) sera; and intrinsic asthma in 63% (5 out of 8) sera. In contrast, there was a much lower proportion of IgM ANA in silicosis in 37% (7 out of 19) sera.

The statistical analysis (Statistical Table 6b) confirmed that the amount of IgM ANA in silicosis was significantly less than in all the other lung diseases except intrinsic asthma.

IgA ANA: The highest proportions of ANA positive sera with IgA ANA (Table 9) were in pleuropulmonary SLE in 33% (9 out of 27) sera, and in cryptogenic fibrosing alveolitis in 30% (9 out

of 30) sera. The proportions of IgA ANA in the other diseases were similarly low, in silicosis 16% (3 out of 19) sera; in asbestosis 14% (3 out of 21) sera and in intrinsic astham 13% (1 out of 8) sera.

These differences were not significant in the statistical tests (Statistical Table 6c).

The Influence of Sex

Differences in the proportions of ANA in any one immunoglobulin class were related to sex in some, but not all instances. (The results are shown in Table 10, and Statistical Table 7, Appendix).

IgG ANA: The lower amount of IgG ANA in asbestosis than the other lung diseases was confirmed, and was not related to sex.

Asbestosis was mainly a male group, and the proportion of IgG ANA in the males 63% (12 out of 19), was significantly lower than in males with pleuropulmonary SLE 100% (7 out of 7); males with cryptogenic fibrosing alveolitis 100% (10 out of 10) and males with silicosis 89% (16 out of 18). It was also lower than in males with intrinsic asthma 100% (4 out of 4) but this difference could not be confirmed for such small numbers. (Table 10 and Statistical Table 7a).

TABLE 10

A comparison of the proportions of the total immunofluorescent ANA positive sera with IgG, IgM and IgA ANAs in the different sexes.

Disease	Sex	Total no. of ANA positive sera	Number positive with antihuman IgG		IgM		IgA	
			No.	%	No.	%	No.	%
Pleuropulmonary	M	7	7	100	3	43*	0	0
SLE	F	20	19	95	17	85	9	45
CFA	M	10	10	100*	6	60	5	50
	F	20	13	65	15	75	4	20
Asbestosis	M	19	12	63**	14	74	3	16
	F	2	1	50	1	50	0	0
Silicosis	M	18	16	89	6	33	3	17
	F	1	1	100	1	100	0	0
Intrinsic asthma	M	4	4	100	2	50	0	0
	F	4	4	100	3	75	1	25
Total patients	M	58	49	84	31	53*	11	19
	F	47	38	81	37	79	14	30

*Significant difference between males and females.
**Significantly lower proportion of IgG ANA than males with all other diseases except intrinsic asthma.
(For the detailed statistical analysis please refer to Appendix , Stat. Table 7).
N.B. % to nearest whole number

Females with cryptogenic fibrosing alveolitis had significantly less IgG ANA 65% (13 out of 20) than the males 100% (10 out of 10); $p < 0.05$. In contrast, the proportion of IgG ANA in males and females with pleuropulmonary SLE and intrinsic asthma was similarly high. (Table 10 and Statistical Table 7d).

IgM ANA: The proportion of sera with IgM ANA was generally lower in males than in females, and taking this into account it could not be confirmed that there is less IgM ANA in silicosis than in the other lung diseases.

Silicosis was mainly a male group, and the proportion of IgM ANA in the males 33% (6 out of 18) was not significantly different from that in males with pleuropulmonary SLE 43% (3 out of 7); males with intrinsic asthma 50% (2 out of 4) or males with cryptogenic fibrosing alveolitis 60% (6 out of 10). However, males with asbestosis did have significantly more IgM ANA 74% (14 out of 19), than those with silicosis; $p < 0.02$. (Table 10 and Statistical Table 7b).

In pleuropulmonary SLE, cryptogenic fibrosing alveolitis and intrinsic asthma the amount of IgM ANA was greater in females than in males, but significantly higher only in pleuropulmonary SLE in 85% (17 out of 20) females compared with 43% (3 out of 7) males; $p < 0.05$. (Table 10 and Statistical Table 7d).

IgA ANA: IgA ANA was seen in males and females but the numbers were not sufficient to make definite conclusions about the influence of sex.

In pleuropulmonary SLE, IgA ANA was present only in females 45% (9 out of 20) not in males (0 out of 7) suggesting a relationship to the female sex. This was not confirmed by the statistical tests. (Table 10 and Statistical Table 7c).

In cryptogenic fibrosing alveolitis IgA ANA was present in 50% (5 out of 10) of males and 20% (4 out of 20) of females; not significantly different. (Table 10 and Statistical Table 7d).

The Influence of Age

The results are shown in Table 11. (Information with regard to age was missing in a few instances).

There was no relationship between the production of IgG, IgM and IgA ANA and the age of the patients.

B. The Profile of IgG, IgM and IgA ANA in ANA Positive Sera in the Different Lung Diseases

The various profiles of IgG, IgM and IgA ANA in ANA positive sera in the different lung diseases are shown and compared in Figure 7 with statistical comparisons in Statistical Table 8, Appendix.

TABLE 11

The relationship between age and immunoglobulin type of ANA. (Statistical comparisons using an unpaired Student t test).

Age distributions for the total sera with:-

IgG ANA			IgM ANA			IgA ANA		
Mean age	SD	n	Mean age	SD	n	Mean age	SD	n
52	13	82	55	12	67	52	14	24

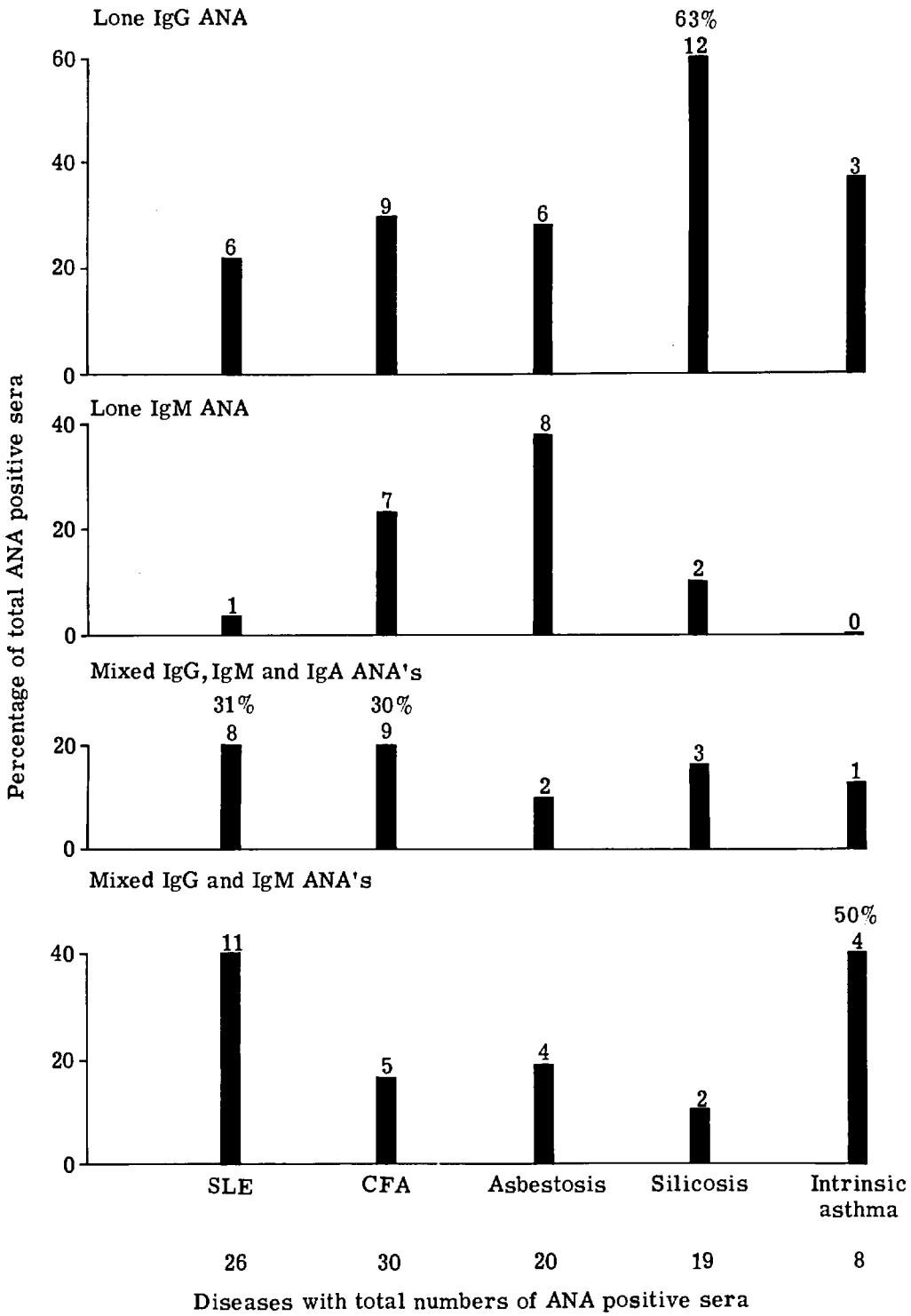
	Significant difference P ≤	
Comparing mean ages ⟶	IgM ANA	IgA ANA
↓		
IgG ANA	NS	NS
IgM ANA		NS

NS = Not significantly different

Figure 7.

Figure showing differences in the numbers and types of immunoglobulin classes of ANA (ANA immunoglobulin class profiles) in individual ANA positive sera in the different lung diseases.

(Actual numbers of sera with the various lone or mixtures of immunoglobulin classes of ANA are indicated at the head of each column)



There were seven possible profiles of IgG, IgM and IgA ANA but only four were common. These were IgG ANA occurring alone; IgM ANA occurring alone; IgG in combination with IgM ANA; and IgG in combination with IgM and IgA ANA.

Of the total 105 ANA positive sera 34% (36 out of 105) contained IgG ANA alone, 17% (18 out of 105) IgM ANA alone, 25% (26 out of 105) IgG combined with IgM ANA, and 22% (23 out of 105) IgG combined with IgM and IgA ANA. IgA ANA never occurred alone in any serum, and nearly always was combined with IgG and IgM ANA. There were just two exceptions, one serum with IgA combined with IgG ANA, and another serum with IgA combined with IgM ANA.

IgG ANA only: There were more sera containing lone IgG ANA in silicosis 63% (12 out of 19) than any other lung disease. Intrinsic asthma had 38% (3 out of 8) sera with lone IgG ANA, cryptogenic fibrosing alveolitis 30% (9 out of 30); asbestosis 29% (6 out of 21) and pleuropulmonary SLE 22% (6 out of 27) (Figure 7a).

The statistical analysis confirmed that there were significantly more sera with lone IgG ANA in silicosis than in the other lung diseases (Statistical Table 8a).

IgM ANA only: The majority of sera with lone IgM ANA were in asbestosis 38% (8 out of 21) sera, and cryptogenic fibrosing alveolitis 23% (7 out of 30) sera. In the remaining diseases, silicosis had 11% (2 out of 19) sera with lone IgM ANA, and pleuropulmonary SLE 4% (1 out of 27). This profile was not seen in intrinsic asthma (Figure 7b).

The statistical analysis confirmed that asbestosis had significantly more sera with lone IgM ANA than any other lung disease, except cryptogenic fibrosing alveolitis. However, the tendency for cryptogenic fibrosing alveolitis to contain more sera with lone IgM ANA than the other diseases, except asbestosis, could only be confirmed in comparison with pleuropulmonary SLE (Statistical Table 8b).

IgG combined with IgM and IgA ANA: Sera with a combination of IgG, IgM and IgA ANA were mainly in pleuropulmonary SLE 30% (8 out of 27), and cryptogenic fibrosing alveolitis 30% (9 out of 30). In the remaining diseases silicosis had 16% (3 out of 19) sera with this combination, intrinsic asthma 11% (1 out of 8) and asbestosis 10% (2 out of 21) (Figure 7c).

The statistical analysis just confirmed that the proportion of sera with IgG, IgM and IgA ANA was significantly higher in pleuropulmonary SLE and cryptogenic fibrosing alveolitis than in asbestosis. The lower proportion of this profile in silicosis and intrinsic asthma was not confirmed (Statistical Table 8c).

IgG combined with IgM ANA: The combination of IgG with IgM ANA was more common in intrinsic asthma 50% (4 out of 8) sera, and pleuropulmonary SLE 41% (11 out of 27) sera, than in the other lung diseases. In the remaining disease asbestosis had 19% (4 out of 21) sera with this combination; cryptogenic fibrosing alveolitis 17% (5 out of 30); and silicosis 11% (2 out of 19) (Figure 7d).

The statistical analysis confirmed that there were significantly more sera with a combination of IgG and IgM ANA in intrinsic asthma and pleuropulmonary SLE than in the other lung diseases (Statistical Table 8d).

IgG combined with IgA ANA: There was just one serum from a patient with pleuropulmonary SLE, showing this combination.

IgM combined with IgA ANA: There was just one serum from a patient with asbestosis showing this combination.

The influence of Sex

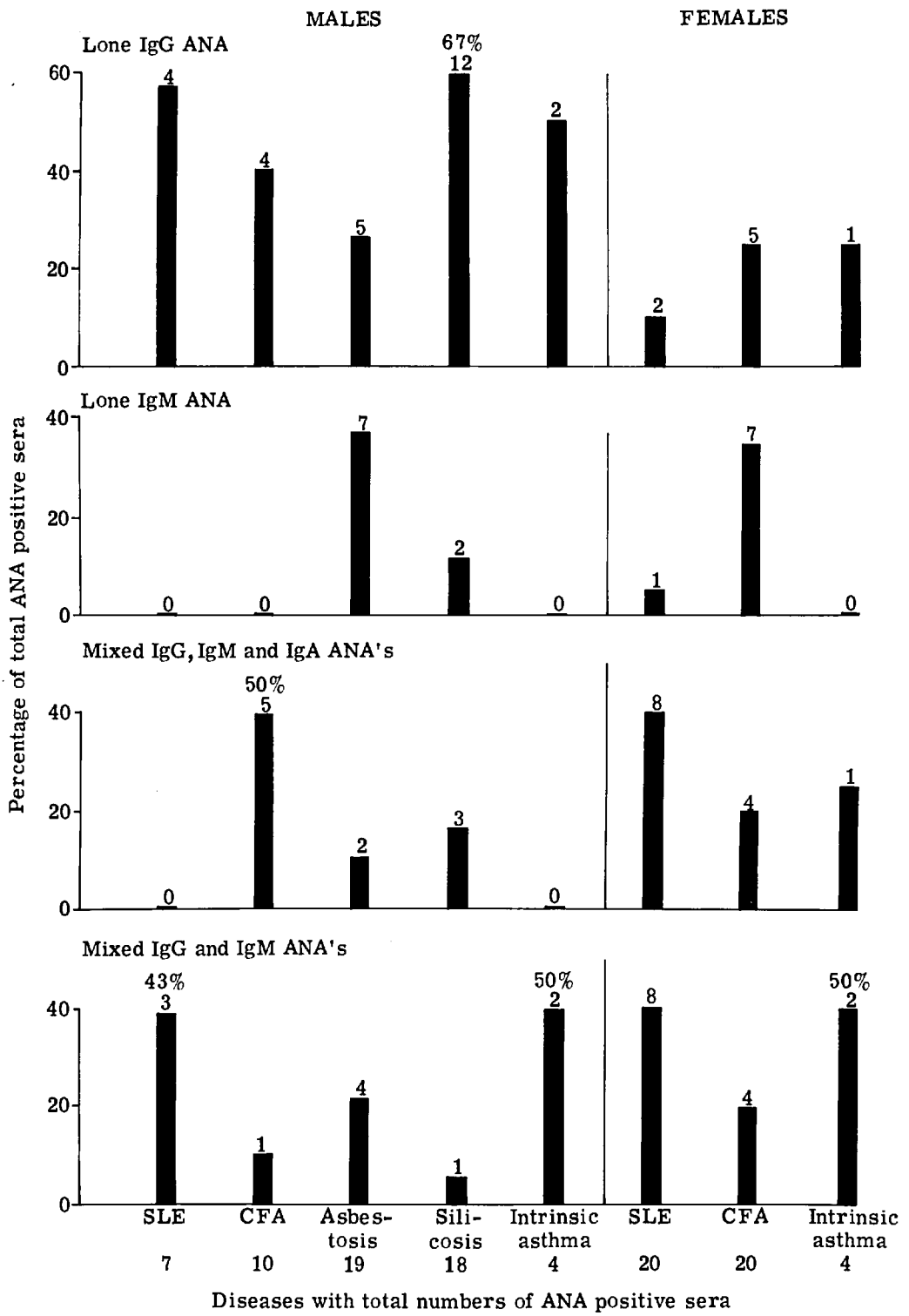
The differences in profiles of immunoglobulin classes of ANA in the different lung diseases were in some cases related to sex differences rather than disease differences. The results are shown in Figure 8 and Statistical Table 9, Appendix.

IgG ANA only: Taking sex into account it could not be confirmed that silicosis has a higher proportion of sera with lone IgG ANA than the other lung diseases, (Figure 8a and Statistical Table 9a). Silicosis was mainly a male group, and when compared with males in most other diseases the proportions of sera with lone IgG ANA were similarly high, in silicosis 67% (12 out of 18) in pleuropulmonary SLE 57% (4 out of 7), in intrinsic asthma 50% (2 out of 4), in cryptogenic fibrosing alveolitis 40% (4 out of 10). However, males with asbestosis had significantly fewer sera with lone IgG ANA 26% (5 out of 19) than males with silicosis; $p < 0.02$.

Figure 8.

A comparison of the numbers and types of immunoglobulin classes of ANA in individual ANA positive sera in male and female patients.

(Actual numbers of sera with the various lone or mixtures of immunoglobulin classes of ANA are indicated at the head of each column)



In pleuropulmonary SLE; cryptogenic fibrosing alveolitis, and intrinsic asthma, there were fewer females with lone IgG ANA than males, but the difference was significant only in pleuropulmonary SLE where 10% (2 out of 20) females had lone IgG ANA compared with 57% (4 out of 7) males; $p < 0.01$.

IgM ANA only: The greater number of sera with lone IgM ANA in asbestosis than in other lung diseases was confirmed, and was not a reflection of sex differences (Figure 8b and Statistical Table 9b).

In the male asbestos group 37% (7 out of 19) sera had lone IgM ANA, significantly more than in males from all other diseases. The only other males with lone IgM ANA were in silicosis 11% (2 out of 18). The statistical analysis confirmed some of these differences.

In cryptogenic fibrosing alveolitis lone IgM ANA was seen only in the females 35% (7 out of 20) not in the males (0 out of 10); $p < 0.05$. These females also had more lone IgM ANA than females with pleuropulmonary SLE 5% (1 out of 20): $p < 0.02$, and females with intrinsic asthma (0 out of 4).

IgG combined with IgM and IgA ANA: This combination was seen in both male and female patients and tended to be most common in

females with pleuropulmonary SLE 40% (8 out of 20), and in males with cryptogenic fibrosing alveolitis 50% (5 out of 10) (Figure 8c).

The statistical analysis confirmed only that there were significantly more males with this combination in cryptogenic fibrosing alveolitis 50% (5 out of 10) than in any other disease (Statistical Table 9c).

IgG combined with IgM ANA: The combination of IgG and IgM ANA was again more common in pleuropulmonary SLE and intrinsic asthma than in the other diseases for both males and females (Figure 8d and Statistical Table 9d).

For the males, the combination was more common in pleuropulmonary SLE 43% (3 out of 7) and intrinsic asthma 50% (2 out of 4), than in cryptogenic fibrosing alveolitis 10% (1 out of 10) silicosis 6% (1 out of 18) and asbestosis 21% (4 out of 19) but the statistical confirmation was incomplete.

The numbers of females with this combination were greater in pleuropulmonary SLE 40% (8 out of 20), and intrinsic asthma 50% (2 out of 4) than in cryptogenic fibrosing alveolitis 20% (4 out of 20) but these differences were not confirmed significant.

The Influence of Age

The results are shown in Table 12. (Information with regard to age was missing in a few instances).

Lone IgM ANA tended to occur in an older age range of patients than the other combinations. The mean age of 18 patients with lone IgM ANA was 61 years SD 9 years; and was significantly higher than that of 33 patients with lone IgG ANA 51 years SD 13 years ($p < 0.02$), 22 patients with IgG, IgM and IgA ANA 52 years SD 14 years ($p < 0.1$), and 26 patients with IgG and IgM ANA 54 years SD 12 years ($p < 0.1$). (This observation is in keeping with the age associated increasing prevalence of ANA in asbestosis with lone IgM ANA the most common finding, and the finding of lone IgM ANA in normal controls).

CORRELATION BETWEEN THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA AND THE ANA TITRE (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN) IN ANA POSITIVE SERA

The results are shown in Table 13 with a statistical analysis in Statistical Table 10, Appendix.

A general pattern of the IgG, IgM and IgA ANA combination relating to a higher range of ANA titres; the IgG, IgM ANA combination and

TABLE 12

The relationship between age and immunoglobulin class profiles of ANA. (Statistical comparisons using an unpaired Student t test).

Age distributions for the total sera with ANA of immunoglobulin class:-

IgG only			IgM only			IgG/IgM/IgA			IgG/IgM		
Mean age	SD	n	Mean age	SD	n	Mean age	SD	n	Mean age	SD	n
51	13	33	61	9	18	52	14	22	54	12	26

	Significant difference $P \leq$		
Comparing mean ages ⟶	IgM only	IgG/IgM/IgA	IgG/IgM
↓			
IgG only	0.02	NS	NS
IgM only		0.1	0.1
IgG/IgM/IgA			NS

NS = Not significantly different

TABLE 13

A comparison of the immunofluorescent ANA titre distributions relating to different profiles of immunoglobulin classes of ANA.

Disease	Total no. of ANA pos. sera (n. total)	Mean log. reciprocal immunofluorescent ANA titres for sera with:											
		Lone IgG			Lone IgM			IgG/IgM/IgA			IgG/IgM		
		Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
SLE	26	2.15	0.72	6	1.00	0.00	1	2.54	0.30	8	1.87	0.43	11
CFA	30	1.87	0.44	9	1.30	0.39	7	2.00	0.56	9	1.90	0.30	5
ASB	21	1.21	0.33	7	1.45	0.32	8	2.35	0.64	2	1.45	0.17	4
SIL	19	1.48	0.53	12	1.15	0.21	2	1.90	0.30	3	1.45	0.21	2
AST	8	1.50	0.46	3	0.00	0.00	0	2.20	0.00	1	1.75	0.30	4
Total patients	104	1.63	0.58	37	1.35**	0.34	18	2.21*	0.49	23	1.76	0.37	26

*Significant finding of highest range of ANA titres associated with combined IgG/IgM/IgA ANAs.
**Significant finding of lowest range of ANA titres associated with lone IgM ANA.

(For the detailed statistical comparisons please refer to Appendix , Stat. Table 10).

lone IgG ANA relating to a middle range of ANA titres; and lone IgM ANA relating to a lower range of ANA titres was common to all the lung disease groups.

The most significant finding was the relationship between a combination of IgG, IgM and IgA ANA and higher ANA titres. The mean log reciprocal ANA titre in sera with this combination was 2.21 (1/160), significantly higher than in sera with IgG and IgM ANA 1.76 (between 1/40 and 1/80); sera with lone IgG ANA 1.63 (approximately 1 in 40), and sera with lone IgM ANA 1.35 (approximately 1 in 20); $p < 0.01$.

The mean ANA titre in sera with lone IgM ANA was significantly lower than in sera with IgG, IgM and IgA ANA, and in sera with IgG and IgM ANA ($p < 0.01$.) However, it did not differ significantly from that in sera with lone IgG ANA.

IV. PATTERNS OF ANA IMMUNOFLUORESCENCE

Patterns of ANA immunofluorescence in any serum were recorded separately for each immunoglobulin class of ANA: IgM, IgG and IgA. By comparison, the patterns recorded using polyvalent antihuman immunoglobulin were inaccurate.

Normally, a single pattern of ANA immunofluorescence was recognised in any one immunoglobulin class, but, because many sera contained a mixture of immunoglobulin classes of ANA, different patterns could be present in the same serum sample relating to different immunoglobulin classes of ANA.

Four main patterns of ANA immunofluorescence were seen: the homogeneous pattern; the peripheral pattern; the speckled pattern and the nucleolar pattern. There was some heterogeneity under each of these main descriptive types, but further descriptive subdivision was not possible.

Examples of each main pattern type with a negative control for comparison are shown in Plates 7 to 11 inclusive.

NORMAL CONTROL STUDIES

There were ten ANA positive sera from normal controls without lung disease. Five were males and five females. None had a homogeneous

A homogeneous pattern of nuclear immunofluorescence produced by ANA from the serum of a patient with SLE (BR) on rat liver cell nuclei. This appearance was obtained using an indirect immunofluorescent staining method with a 1 in 10 dilution of patient's serum and FITC-conjugated goat antihuman IgG to visualise the antibody. (Magnification x 480)

A peripheral pattern of nuclear immunofluorescence produced by ANA from the serum of a male control (PR.) on rat liver cell nuclei. This appearance was obtained using an indirect immunofluorescent staining method with a 1 in 10 dilution of test serum and FITC-conjugated goat antihuman IgM to visualise the antibody. (Magnification x 480)

Plate 7

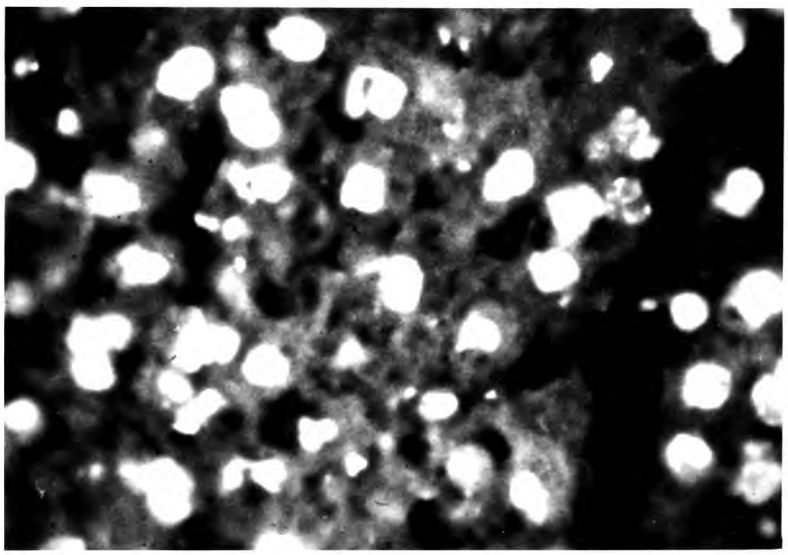
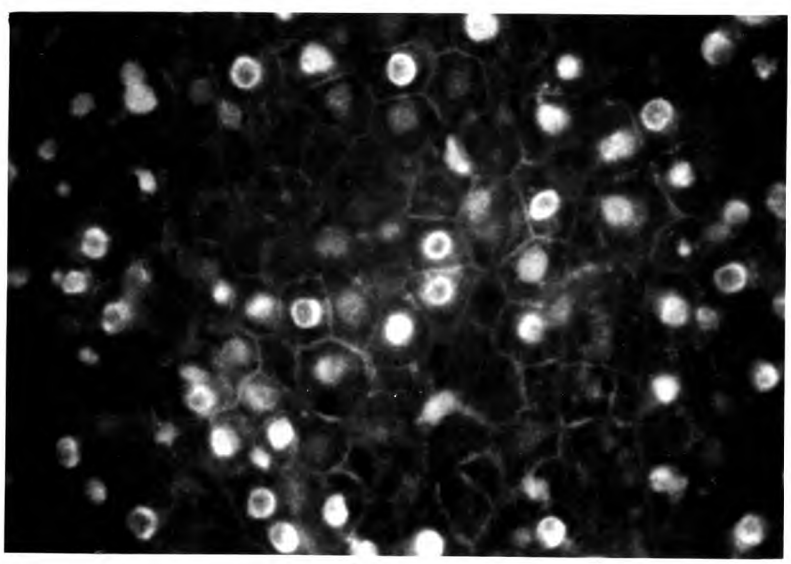


Plate 8



A speckled pattern of nuclear immunofluorescence produced by ANA from the serum of a patient with CFA (OS.) on rat liver cell nuclei. This appearance was obtained using an indirect immunofluorescent staining method with a 1 in 10 dilution of patient's serum and FITC-conjugated goat anti-human IgM to visualise the antibody. (Magnification x 480)

A nucleolar pattern of immunofluorescence produced by ANA from the serum of a patient with CFA (TH.) on rat liver cell nuclei. This appearance was obtained using an indirect immunofluorescent staining method with a 1 in 10 dilution of patient's serum and FITC-conjugated antihuman IgG to visualise the antibody. (Magnification x 300)

Plate 9

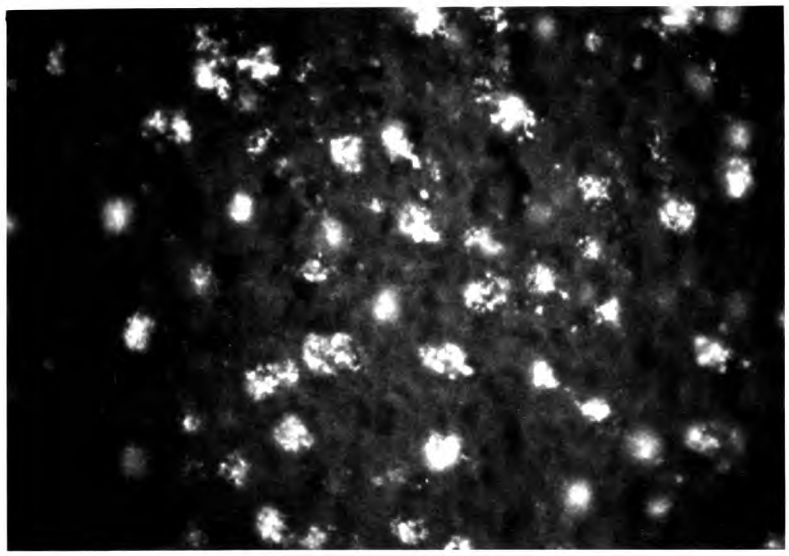
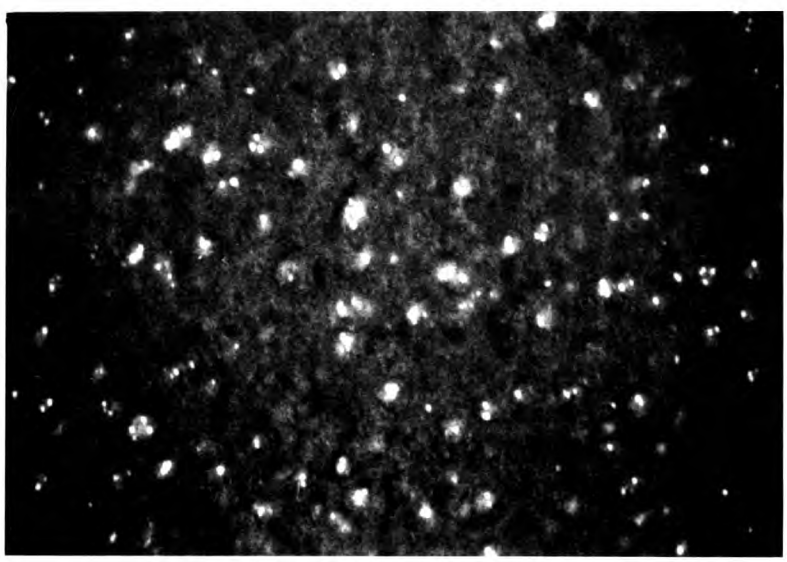
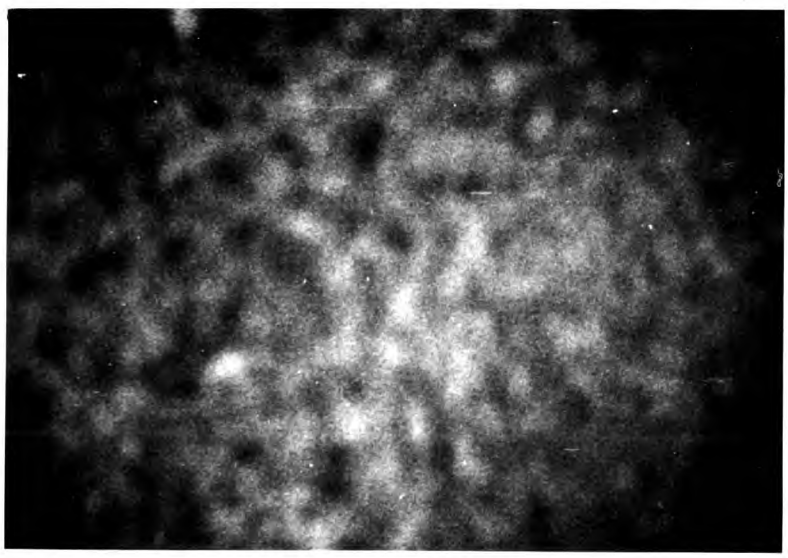


Plate IO



A negative appearance in an indirect immunofluorescent staining test for ANA showing only non-specific background staining of the rat liver cryostat section used as the antigen. This was observed using a 1 in 10 dilution of a normal control serum and FITC-conjugated goat antihuman immunoglobulin as the fluorescent tracer. (Magnification x 300).

Plate II



pattern, 5 (4 males and 1 female) had a peripheral pattern, 4 (3 females and 1 male) had a speckled pattern and 2 (females) had a nucleolar pattern.

The patterns were lone patterns associated with IgM ANA, except in one female who had a combination of a nucleolar pattern associated with IgG ANA and a speckled pattern associated with IgM ANA.

LUNG DISEASE STUDIES

The ANA positive sera used for this investigation were the same as those used to investigate ANA titres and immunoglobulin classes of ANA.

There were sera from 27 patients with pleuropulmonary SLE; 30 patients with cryptogenic fibrosing alveolitis; 21 patients with asbestosis, 19 patients with silicosis and 8 patients with intrinsic asthma.

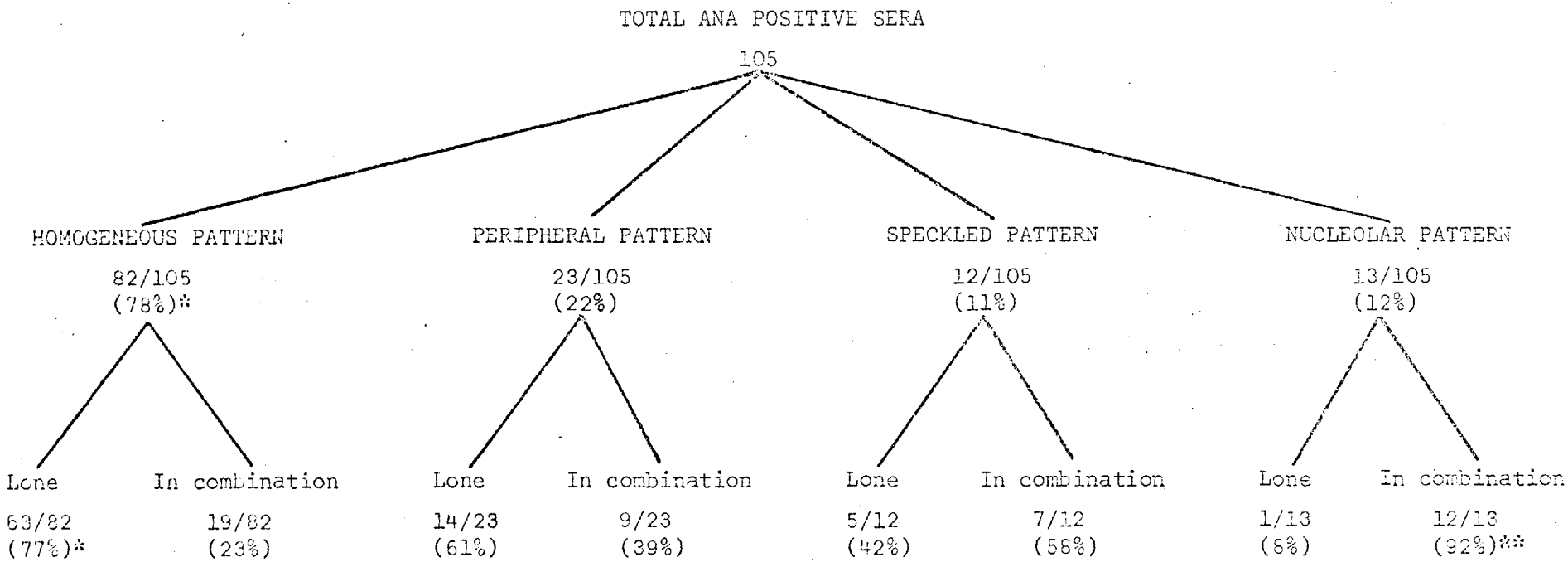
Total Patterns

The results are shown in Figure 9 with statistical comparisons in Statistical Table 11, Appendix.

There were a total of 105 ANA positive sera. The homogeneous pattern

FIGURE 9

The total patterns of ANA immunofluorescence in positive sera (relating to either IgG, IgM or IgA ANA's).



Lone = The only pattern in the serum whatever the Ig class of ANA.

In combination = In combination with other patterns normally in <u>different</u> Ig classes of ANA.

* - Significantly more common than any other pattern, and generally a lone pattern.

** - Significantly occurring in combination with other patterns. (For detailed statistical comparisons refer to Appendix , Stat. Table 11).

was present in significantly more sera 78% (82 out of 105) of the sera, than any other pattern. The peripheral pattern was seen in only 22% (23 out of 105), but still significantly more than the speckled or nucleolar patterns. The nucleolar pattern was seen in only 12% (13 out of 105), and similarly the speckled pattern was seen in only 11% (12 out of 105) (Figure 9 and Statistical Table 11a).

The nucleolar pattern was, with one exception, always combined with other patterns in different immunoglobulin classes in the same serum, 92% (12 out of 13) in combination compared with 8% (1 out of 13) lone; $p < 0.01$. In contrast the homogeneous peripheral and speckled patterns were frequently the only patterns present in the serum, and also occurred in combination with other patterns. The homogeneous pattern was significantly more frequent as a lone pattern, 77% (63 out of 82) of cases, than as a combined pattern, 23% (19 out of 82) of cases; $p < 0.01$. However, it was nearly always a partner in any combination in view of its more common occurrence overall (Figure 9 and Statistical Table 11b).

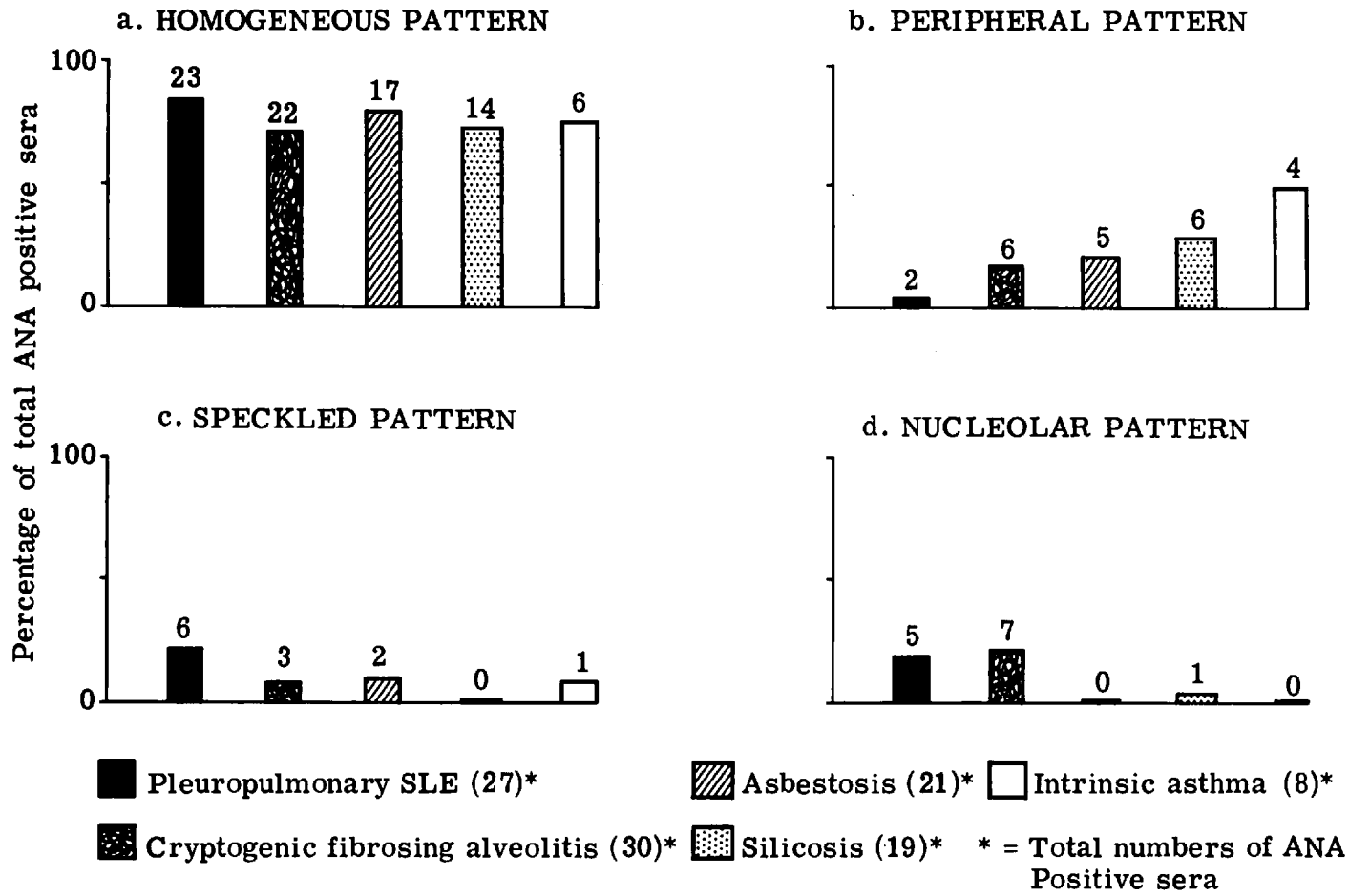
Relation to Disease

The results are shown in Figure 10 with statistical comparisons in Statistical Table 12, Appendix.

The homogeneous pattern was the most common staining pattern in all disease groups and the proportions did not differ significantly.

Figure 10.

A comparison of the patterns of ANA immunofluorescence in the different lung diseases. (Actual numbers of sera with the patterns in each disease group are indicated at the head of the columns).



It was present in 85% (23 out of 27) of sera with pleuropulmonary SLE, 81% (17 out of 21) with asbestosis, 75% (6 out of 8) with intrinsic asthma, 74% (14 out of 19) with silicosis and 73% (22 out of 30) with cryptogenic fibrosing alveolitis (Figure 10a).

The peripheral pattern was more common in silicosis 32% (6 out of 19) sera, and intrinsic asthma 50% (4 out of 8) sera, than in cryptogenic fibrosing alveolitis 20% (6 out of 30) sera and asbestosis 24% (5 out of 21) sera, and was surprisingly uncommon in pleuropulmonary SLE 7% (2 out of 27) sera (Figure 10b).

There was very little of the speckled pattern in any disease group but it was notably absent from silicosis (0 out of 19) sera (Figure 10c).

The nucleolar pattern was present in cryptogenic fibrosing alveolitis 23% (7 out of 30) sera and in pleuropulmonary SLE 19% (5 out of 27) sera but, except for one patient with silicosis, was absent from the other diseases (Figure 10d).

These relationships were indicated in the statistical tests but only partially confirmed because of the small numbers (Statistical Table 12 a, b, c, d).

The Influence of Sex

The results are shown in Figure 11.

The homogeneous, peripheral, speckled and nucleolar patterns were seen in males as well as in females. There was a slight tendency for the speckled and nucleolar patterns to be more common in females than males.

A statistical analysis confirmed only that the speckled pattern was significantly more common in females than males; $p < 0.02$.

The Influence of Age

There was no significant relationship between differences in the patterns of ANA immunofluorescence and age.

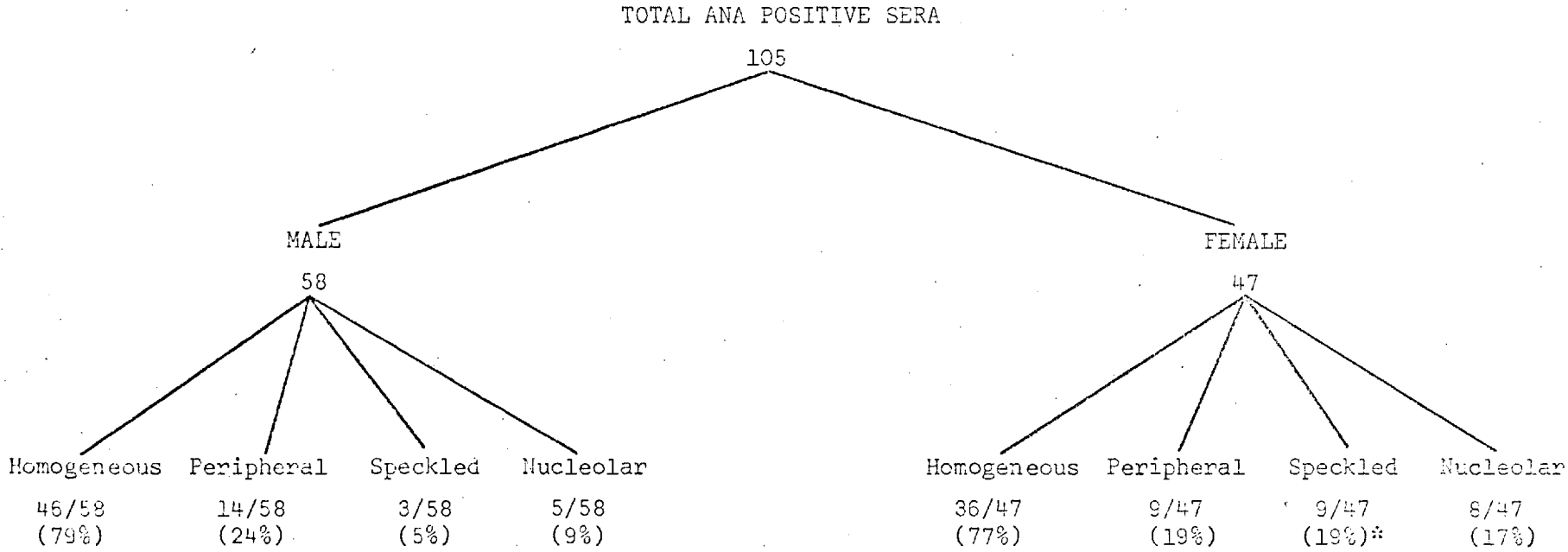
CORRELATION BETWEEN PATTERNS OF ANA IMMUNOFLUORESCENCE AND THE ANA TITRE (USING POLYVALENT ANTIHUMAN IMMUNOGLOBULIN) IN ANA POSITIVE SERA

The results are shown in Table 14 including statistical comparisons.

The nucleolar pattern was associated with a significantly higher range of ANA titres, mean log reciprocal titre 2.12 (approximately 1/160), than the peripheral pattern, 1.72 (1/40 - 1/80) and the

FIGURE 11

A comparison of the patterns of ANA immunofluorescence in male and female sera.



*Speckled pattern significantly more common in females than in males; $p < 0.02$ (using a test of difference of proportions

TABLE 14

A comparison of the immunofluorescent ANA titre distributions relating to different patterns of ANA immunofluorescence.

	Mean log. reciprocal immunofluorescent ANA titre for sera with:											
	Homogeneous pattern			Peripheral pattern			Speckled pattern			Nucleolar pattern		
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Pattern occurring alone	1.65	0.55	63	1.43	0.31	14	1.72	0.45	5	2.20	0	1
Pattern in combination with other patterns	2.02*	0.55	19	1.90*	0.50	9	2.20	0.42	7	2.17	0.53	12
Total alone or in combination	1.73	0.57	82	1.72	0.49	23	2.00	0.48	12	2.12**	0.49	13

**Nucleolar pattern associated with a significantly higher range of ANA titres than homogeneous or peripheral patterns ($p < 0.05$).

*Significantly higher range of ANA titres for combination than 'lone' pattern ($p < 0.1$).

SD - Standard deviation
n - Number of sera

(Statistical comparisons using an unpaired Student t test)

homogeneous pattern, 1.73 (1/40 - 1/80). The speckled pattern was associated with a mid range of ANA titres 2.00 (1/80 - 1/60) which did not differ significantly from any other pattern.

The nucleolar pattern was, however, nearly always in combination with other patterns in the same serum, and in fact, all sera with a combination of immunofluorescent patterns, whatever the patterns, were associated with a similarly high range of ANA titres (mean titre 1/80 - 1/160 approximately). In contrast, sera containing a single pattern of ANA immunofluorescence; a lone homogeneous, a lone peripheral or a lone speckled; were associated with a similarly low range of ANA titres (mean titre 1/40 approximately).

CORRELATION BETWEEN PATTERNS OF ANA IMMUNOFLUORESCENCE AND IMMUNOGLOBULIN CLASSES OF ANA

Relationship to Immunoglobulin Class of ANA

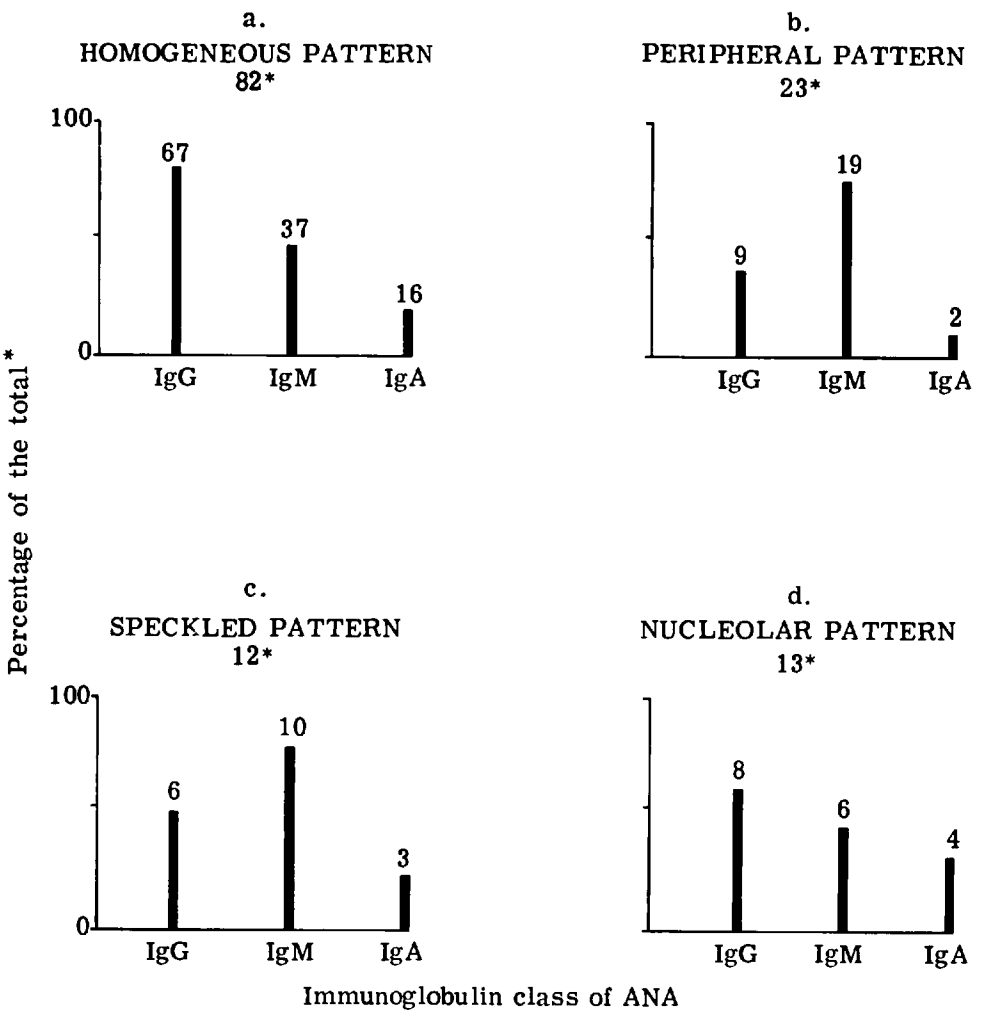
The results are shown in Figure 12 with statistical comparisons in Statistical Table 13, Appendix.

The different staining patterns were found to relate more to certain immunoglobulin classes of ANA than others, although all four main patterns were seen in each of the three immunoglobulin classes of ANA.

The homogeneous pattern was related significantly more to IgG ANA than IgM or IgA ANA, and significantly least to IgA ANA.

Figure 12.

Figure to show the tendency for different staining patterns of immunofluorescent ANA to be more common in certain immunoglobulin classes of ANA than others.



* = Total numbers of sera with pattern in any immunoglobulin class. (The actual numbers with the pattern in each immunoglobulin class are indicated at the head of each column).

82% (67 out of 82) of the total sera with a homogeneous pattern were observed in IgG, in comparison with only 45% (37 out of 82) in IgM and 20% (16 out of 82) in IgA (Figure 12a and Statistical Table 13a).

In contrast, the peripheral and speckled patterns were related more to IgM ANA than IgG or IgA ANA, and least to IgA ANA (figure 12 b and c).

82% (19 out of 23) of the total sera with a peripheral pattern were observed in IgM in comparison with 39% (9 out of 23) in IgG and 9% (2 out of 23) in IgA. 83% (10 out of 12) of the total sera with speckled pattern were observed in IgM, in comparison with 50% (6 out of 12) in IgG and 25% (3 out of 12) in IgA. Most of these differences were confirmed by the statistical tests (Statistical Table 13 b and c).

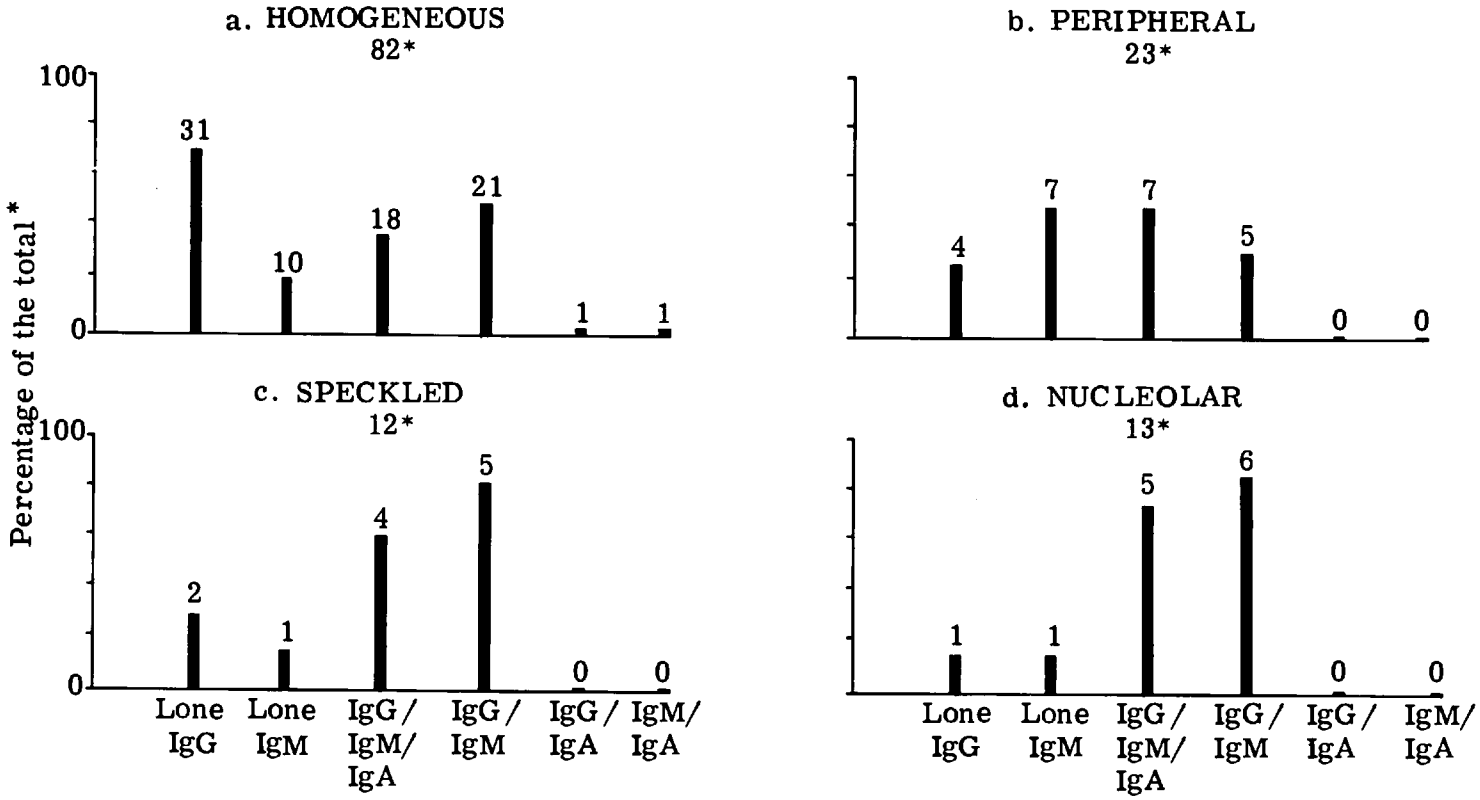
The nucleolar pattern was not convincingly related to any one immunoglobulin class. Slightly more of the total were observed in IgG 62% (8 out of 13), than in IgM 46% (6 out of 13) or IgA 31% (4 out of 13), but these differences were not significant (Figure 12d and Statistical Table 13d).

Relationship to the Profile of IgG, IgM and IgA ANA in Individual Sera

The detailed results are shown in Figure 13.

Figure 13.

A comparison of the patterns of ANA immunofluorescence and the profiles (number and types) of immunoglobulin classes of ANA in individual sera.



Profile (number and types) of immunoglobulin classes of ANA in individual sera

* = Total number of sera with the pattern in any immunoglobulin class. (Actual numbers with each profile are indicated at the head of each column).

In summary, the homogeneous and peripheral patterns were seen in sera with lone immunoglobulin classes of ANA, and in those with combined immunoglobulin classes of ANA.

In contrast, the speckled and nucleolar patterns were significantly more frequent in sera with combined immunoglobulin classes of ANA.

V. COMPLEMENT FIXING ABILITY OF ANA

The immunofluorescent indirect staining method used to determine the ability of antinuclear antibodies to fix fresh human serum complement was very satisfactory. There was no difficulty in identifying positive reactions which resembled the normal immunofluorescent ANA appearance, although distinct differences in staining patterns were generally not clearly recognizable.

Examples of complement fixing ANA using the immunofluorescent technique with a negative control for comparison are shown in Plates 12, 13 and 14.

Reactions seen using a standard serum dilution of 1 in 10 and graded + or over were accepted as positive, and weak reactions graded $\pm$ were regarded as negative for counting purposes to reduce inter-observer error.

NORMAL CONTROL STUDIES

The ten ANA positive sera (5 males and 5 females) from normal controls without lung disease did not have complement fixing ANA.

LUNG DISEASE STUDIES

The ANA positive sera used for this investigation were the same as those used to investigate ANA titres, immunoglobulin classes of ANA and patterns of ANA immunofluorescence. The differences in numbers

Immunofluorescent demonstration of complement fixed to ANA from the serum of a patient with SLE (CO.) on rat liver cell nuclei. The method used is an indirect immunofluorescent staining method with fresh normal human serum (PH.) as a source of active complement and FITC-conjugated horse antihuman βiC/A + βiE to visualise the bound complement. (Magnification x 756)

Immunofluorescent demonstration of complement fixed to nucleolar ANA from the serum of a patient with CFA (SM.) on rat liver cell nuclei using the indirect immunofluorescent staining method. (Magnification x 756)

Plate I2

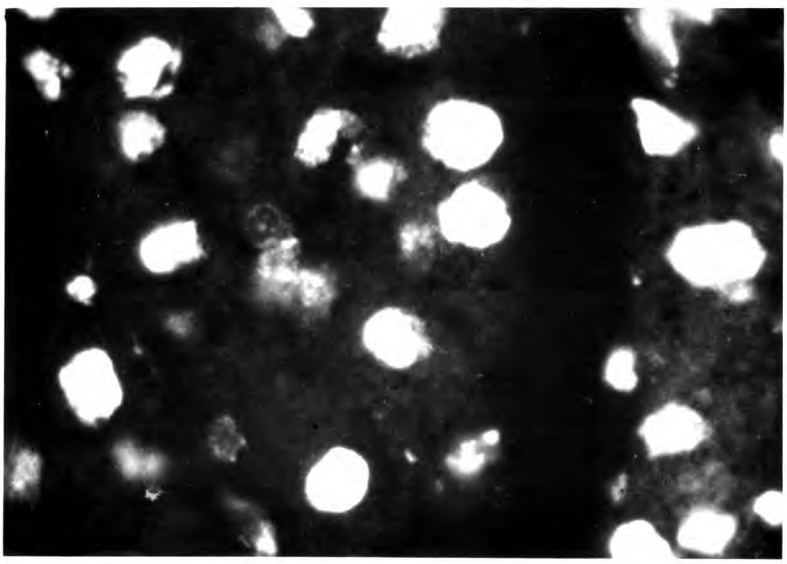
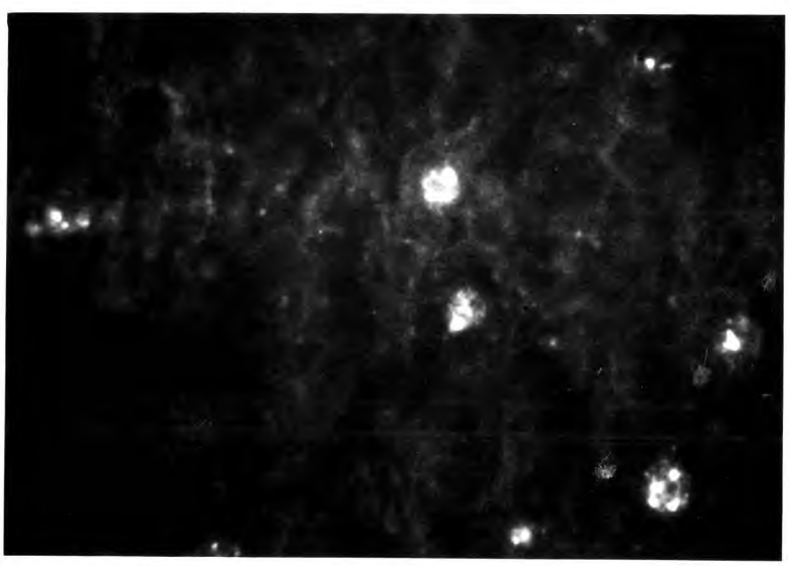
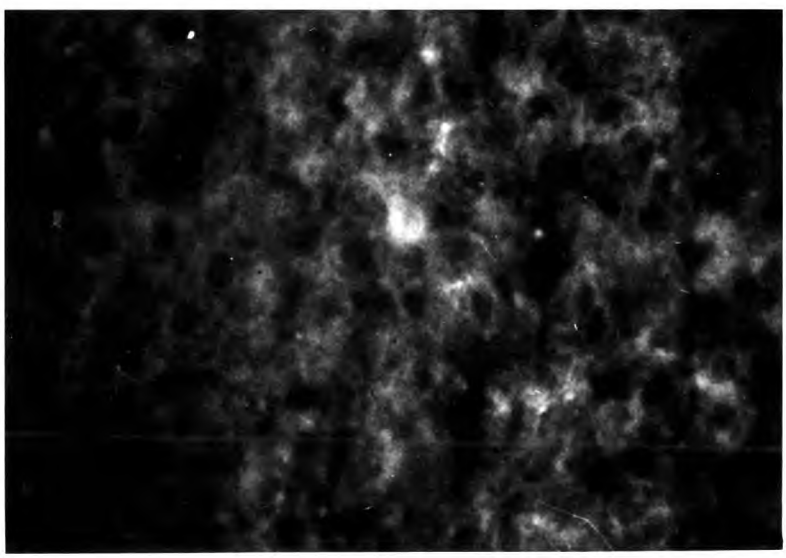


Plate I3



A negative result in the indirect immunofluorescent test for complement fixing ANA. The test serum was from a patient (BR.) with SLE, which had given strongly positive ANA immunofluorescence using FITC-conjugated goat antihuman IgG (Plate 7). (Magnification x 480)

Plate I4



related to insufficient serum in some instances.

There were a total of 133 ANA positive sera from twenty-eight patients with pleuropulmonary SLE, fifty patients with cryptogenic fibrosing alveolitis, twenty-four patients with asbestosis, twenty-two patients with silicosis and nine patients with intrinsic asthma.

The proportion of the total ANA positive sera containing ANA with the ability to fix fresh human complement in each lung disease group is shown in Table 15, with statistical comparisons in Statistical Table 14, Appendix.

Complement fixing ANA's were comparatively rare in lung disease, present in only 31 (23%) of the total 133 ANA positive sera. There was an obvious disease relationship: ANA's with the ability to fix complement were present in pleuropulmonary SLE in 39% (11 out of 28) of sera, and in cryptogenic fibrosing alveolitis in 34% (17 out of 50) of sera. In contrast, they were rare in silicosis seen in only 3 (14%) of the 22 sera, and were completely absent from asbestosis (0 out of 24 sera) and intrinsic asthma (0 out of 9 sera).

The statistical analysis (Statistical Table 14) confirmed that the proportions of complmenet fixing ANA in pleuropulmonary SLE and cryptogenic fibrosing alveolitis were similar, and were significantly increased in comparison with all other lung diseases.

TABLE 15

The proportion of immunofluorescent ANA positive sera containing ANA with the ability to fix human complement.

Disease	Total number of ANA positive sera	Number with complement fixing ANA	
		No.	%
Pleuropulmonary SLE	28	11	39*
CFA	50	17	34*
Asbestosis	24	0	0
Silicosis	22	3	14
Intrinsic asthma	9	0	0
Total patients	133	31	23

*Significant increases in comparison with the other diseases (For the detailed statistical analysis, please refer to Appendix , Stat. Table 14).

In addition, the slight increase in complement fixing ANA in silicosis was significant in comparison with asbestosis ($p < 0.1$) but not with intrinsic asthma.

The Influence of Sex

The results are shown in Table 16 with statistical comparisons in Statistical Table 15, Appendix.

There was a suggestion that complement fixing ANA might relate to sex, and not to disease, but this could not be confirmed with certainty because of the small numbers, and the absence of female patients from asbestosis and silicosis.

Complement fixing ANA was seen in males and in females, but the frequency was higher in females than in males. In pleuropulmonary SLE 10 (48%) out of 21 females had complement fixing ANA in comparison with only 1 (14%) out of 7 males. In cryptogenic fibrosing alveolitis 12 (41%) out of 29 females had complement fixing ANA in comparison with only 5 (24%) out of 21 males.

The statistical analysis could not confirm that the increased frequency of complement fixing ANA in females was significantly above that in males with pleuropulmonary SLE and cryptogenic fibrosing alveolitis (Statistical Table 15a).

However, nor could it confirm that there was a significant increase

TABLE 16

A comparison of the proportions of immunofluorescent ANA positive sera containing complement fixing ANA in the different sexes.

Disease	Sex	Total number of ANA positive sera	Number with complement fixing ANA	
			No.	%
Pleuropulmonary SLE	M	7	1	14
	F	21	10	48
CFA	M	21	5	24
	F	29	12	41
Asbestosis	M	18	0	0
	F	2	0	0
Silicosis	M	21	3	14
	F	1	0	0
Intrinsic asthma	M	5	0	0
	F	4	0	0
Total patients	M	72	9	12
	F	57	22	39.5

in the frequency of complement fixing ANA in males with pleuropulmonary SLE and cryptogenic fibrosing alveolitis over the frequency in males from any other disease group with the exception of asbestosis (Statistical Table 15b).

The male asbestos group was shown to have significantly less complement fixing ANA than males with silicosis or males with cryptogenic fibrosing alveolitis.

The Influence of Age

There was no relationship between complement fixing ability and age.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND ANA TITRES IN ANA POSITIVE SERA

The results are shown in Table 17 with statistical comparisons.

Complement fixing ANA's were associated with a higher range of ANA titres than non-complement fixing ANA's. Overall, 94% (29/31) of sera with complement fixing ANA had ANA titres $\geq$ 1/40 and 68% (10/31) titres $\geq$ 1/80, in contrast with only 50% (51/102) and 33% (34/102) respectively of those without complement fixing ANA; $p < 0.001$. The mean log. reciprocal ANA titre for the total 31 sera with complement fixing ANA was 2.09 (between 1/80 and 1/160), significantly higher than the mean for the total 102 sera with non-complement fixing ANA 1.54 (between 1/20 and 1/40); $p < 0.001$.

TABLE 17

A comparison of the immunofluorescent ANA titre distributions in sera with and without complement fixing ANA.

Disease	Complement fixing ANA	Total No. of ANA pos. sera	Number and % of total sera at each titre														†Mean ANA titre	S.D.	*Sig. diff. P ≤
			Reciprocal of ANA titre																
			10		20		40		80		160		320		640				
			Logarithm of reciprocal of ANA titre																
			1.0		1.3		1.6		1.9		2.2		2.5		2.8				
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	Present	11	0	0	0	0	2	18	0	0	4	36	3	27	2	18	2.28	0.40	NS
	Absent	17	4	24	0	0	3	18	1	6	4	24	1	6	4	24	1.95	0.68	
CFA	Present	17	0	0	2	12	5	29	3	18	4	24	1	6	2	12	1.95	0.46	0.01
	Absent	33	14	42	3	9	5	15	8	24	2	6	0	0	1	3	1.46	0.48	
ASB	Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
	Absent	24	4	17	9	37	5	21	5	21	0	0	0	0	1	4	1.50	0.41	
SIL	Present	3	0	0	0	0	1	33	0	0	1	33	0	0	1	33	2.20	0.60	0.01
	Absent	19	6	32	8	42	3	16	1	5	1	5	0	0	0	0	1.33	0.33	
AST	Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
	Absent	9	2	22	1	11	1	11	4	44	1	11	0	0	0	0	1.63	0.43	
Total patients	Present	31	0	0	2	6	8	26	3	10	9	29	4	13	5	16	2.09	0.46	0.001
	Absent	102	30	29	21	21	17	17	19	19	8	8	1	1	6	6	1.54	0.51	

*Statistical comparisons using an unpaired Student t test.
†Log. reciprocal.

N.B. % to nearest whole number.

Considering the three disease groups with complement fixing ANA separately; the association could not be seen in pleuropulmonary SLE where the ANA titres were similarly high in sera with and without complement fixing ANA. The mean log. reciprocal ANA titre for the 11 sera with complement fixing ANA was 2.28 (approximately 1/160), slightly but not significantly higher than the mean for the 17 sera with non-complement fixing ANA 1.95 (approximately 1/80). In cryptogenic fibrosing alveolitis the mean ANA titre for the 17 sera with complement fixing ANA was 1.95 (approximately 1/80), significantly higher than the mean for the 33 sera with non-complement fixing ANA 1.46 (between 1/20 and 1/40); $p < 0.01$. In silicosis the mean ANA titre for the 3 sera with complement fixing ANA was 2.20 (1/160), significantly higher than that for the 19 with non-complement fixing ANA 1.33 (1/20); $p < 0.01$.

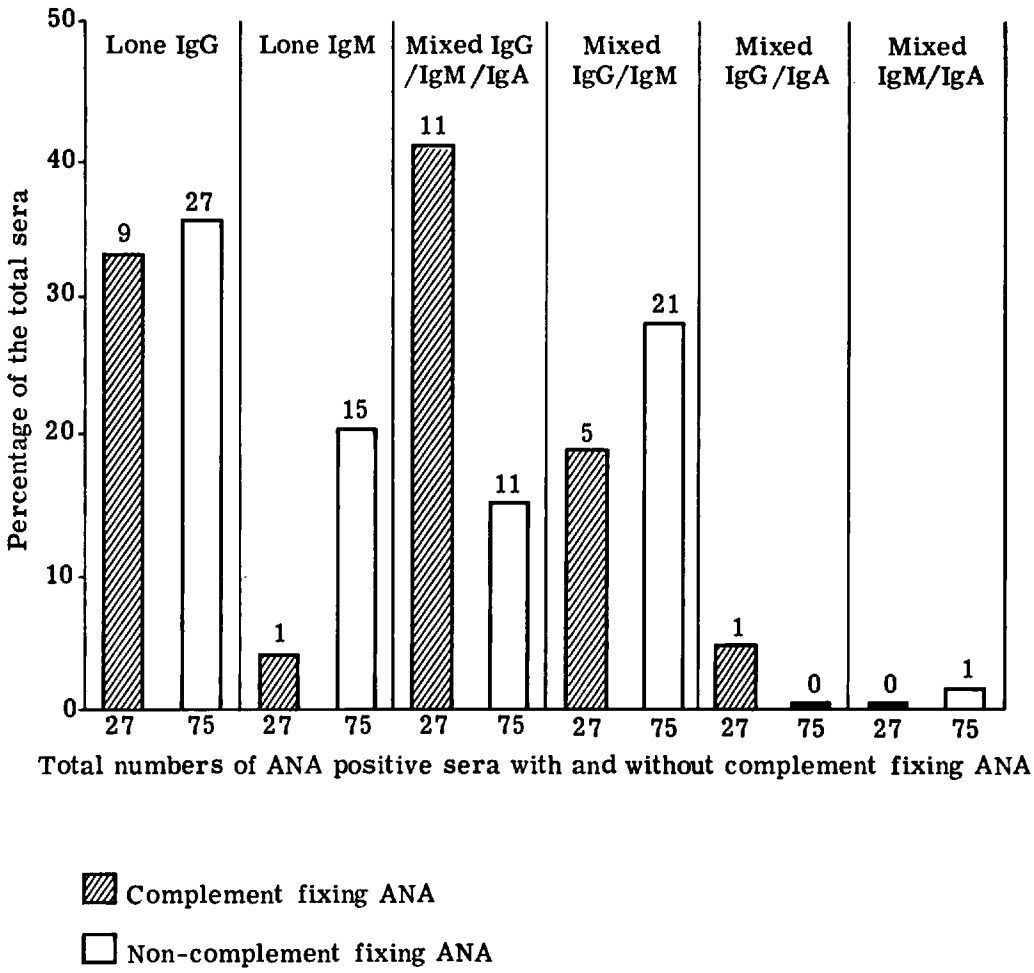
In addition, the mean ANA titres for sera with complement fixing ANA were similar in all disease groups, and were higher than the mean ANA titres for sera with non-complement fixing ANA which were again similar, except in pleuropulmonary SLE.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA

The results are shown in Figure 14 with statistical comparisons in Statistical Table 16, Appendix.

Figure 14.

A comparison of the numbers and types of immunoglobulin classes of ANA in sera with and without complement fixing ANA. (Actual numbers of sera indicated at the head of each column).



There was a relationship between complement fixing ability and the profile of immunoglobulin classes of ANA in individual sera. The immunoglobulin classes of ANA were known for 27 out of the total 31 sera with complement fixing ANA. The majority, 11 (41%) out of 27, were sera with a combination of IgG, IgM and IgA ANA. Slightly fewer, 9 (33%) out of 27 had lone IgG ANA; and fewer still, 5 (19%) out of 27 had combined IgG and IgM ANA. Only 1 (4%) had lone IgM ANA. In addition there was 1 (4%) with a combination of IgG and IgA ANA.

The proportion of sera with IgG, IgM and IgA ANA was significantly higher in sera with complement fixing ANA 41% (11 out of 27), than in those with non-complement fixing ANA 15% (11 out of 75); $p < 0.01$. In addition, the proportion of sera with lone IgM ANA was significantly lower in sera with complement fixing ANA 4% (1 out of 27) than in those with non-complement fixing ANA 36% (27 out of 75); $p < 0.05$.

The detailed results for each separate lung disease group are shown in Table 18.

CORRELATION BETWEEN COMPLEMENT FIXING ABILITY AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA

The results are shown in Table 19 with statistical comparisons

TABLE 18

A comparison of the profiles of immunoglobulin classes of ANA in sera with and without complement fixing ANA.

Disease	Complement fixing ANA	Total no. of sera	Immunoglobulin class profile (No. and % of total sera)											
			Lone IgG		Lone IgM		IgG/IgM/IgA		IgG/IgM		IgG/IgM		IgM/IgA	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pleuropulmonary SLE	Present	11	3	27	0	0	4	36	3	27	1	9	0	0
	Absent	16	3	19	1	6	3	19	9	56	0	0	0	0
CFA	Present	13	4	31	1	7	6	46	2	15	0	0	0	0
	Absent	17	5	29	6	35	3	18	3	18	0	0	0	0
Asbestosis	Present	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	18	6	33	6	33	2	11	3	17	0	0	1	6
Silicosis	Present	3	2	67	0	0	1	33	0	0	0	0	0	0
	Absent	16	10	63	2	12	2	12	2	12	0	0	0	0
Intrinsic asthma	Present	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	8	3	38	0	0	1	12	4	50	0	0	0	0
Total patients	Present	27	9	33	1	4*	11	41**	5	18	1	4	0	0
	Absent	75	27	36	15	20	11	15	21	28	0	0	1	1

*Profile significantly less common in sera with complement fixing ANA than those without; $p < 0.05$.
**Profile significantly more common in sera with complement fixing ANA than those without; $p < 0.01$.
(Statistical comparisons using a test of difference of proportions).
N.B. % to nearest whole number.

in Statistical Table 17 a and b, Appendix .

The speckled and nucleolar immunofluorescent staining patterns were significantly more common in sera with complement fixing ANA than in sera with non-complement fixing ANA. The speckled pattern was seen in 8 (30%) of the total 27 sera with complement fixing ANA, compared with only 3 (4%) of the total 75 sera with non-complement fixing ANA; $p < 0.001$. The nucleolar pattern was seen in 10 (37%) of the total 27 sera with complement fixing ANA, compared with only 3 (4%) of the total 75 sera with non-complement fixing ANA; $p < 0.001$.

There was a very low frequency of the peripheral pattern in sera with complement fixing ANA: The pattern was seen in only 2 (7%) out of the 27 sera with complement fixing ANA, compared with 21 (28%) out of the 75 sera with non-complement fixing ANA; $p < 0.05$.

The homogeneous pattern was seen in 18 (67%) of the 27 sera with complement fixing ANA, and 61 (81%) of the 75 sera with non-complement fixing ANA. The proportion of sera with the homogeneous pattern in combination with other patterns was significantly higher in sera with complement fixing ANA, than in sera with non-complement fixing ANA ($p < 0.1$ where it was more frequently the only pattern in the serum ($p < 0.01$.

TABLE 19

A comparison of the patterns of ANA immunofluorescence in sera with and without complement fixing ANA.

Patterns	Number of sera with complement fixing ANA							Number of sera without complement fixing ANA						
	Total sera		SLE	CFA	ASB	SIL	AST	Total sera		SLE	CFA	ASB	SIL	AST
	27	(100%)	11	13	0	3	0	75	(100%)	16	17	18	16	8
Homogeneous pattern	18	67	8	8	0	2	0	61	81	15	14	14	12	6
Lone pattern	10	37	4	4	0	2	0	50	67	13	11	12	11	3
In combination	8	30	4	4	0	0	0	11	15	2	3	2	1	3
Peripheral pattern	2	7**	1	1	0	0	0	21	28	1	5	5	6	4
Lone pattern	1	4	0	1	0	0	0	12	16	1	3	3	3	2
In combination	1	4	1	0	0	0	0	9	12	0	2	2	3	2
Speckled pattern	8	30*	5	3	0	0	0	3	4	1	0	1	0	1
Lone pattern	3	11	1	2	0	0	0	1	1	0	0	1	0	0
In combination	5	19	4	1	0	0	0	2	3	1	0	0	0	1
Nucleolar pattern	10	37*	3	6	0	1	0	3	4	2	1	0	0	0
Lone pattern	2	7	1	1	0	0	0	0	0	0	0	0	0	0
In combination	8	30	2	5	0	1	0	3	4	2	1	0	0	0

*Speckled and nucleolar patterns significantly more common in sera with complement fixing ANA than those without; $p < 0.001$

**Peripheral pattern significantly less common in sera with complement fixing ANA than those without; $p < 0.05$

(Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number.

VI. ANTIBODIES REACTIVE WITH CALF THYMUS DEOXYRIBONUCLEOPROTEIN

The indirect immunofluorescent staining technique used to examine ANA positive sera for antibodies to deoxyribonucleoprotein (p.94) was subject to considerable inter-observer error for reactions graded $\pm$ and +. These gradations were associated with patchy areas of immunofluorescence of various unusual appearances.

Complete agreement was obtained for positive reactions graded ++ or over, and which were associated with a very characteristic immunofluorescent staining appearance shown in Plate 15. A negative control is shown in Plate 16 for comparison.

To reduce inter-observer error, only reactions graded ++ or over were accepted as positive for counting purposes.

NORMAL CONTROL STUDIES

The ten ANA positive sera (5 males and 5 females) from normal controls without lung disease did not have antibodies to deoxyribonucleoprotein.

LUNG DISEASE STUDIES

The ANA positive sera used for this investigation were the same as those used to investigate ANA titres, immunoglobulin classes of ANA, patterns of ANA immunofluorescence and complement fixing ability of ANA. The differences in numbers related to insufficient serum in some instances.

Immunofluorescent demonstration of deoxyribonucleoprotein antibody from the serum of a patient with SLE (DO.) on a 'spot' of calf thymus deoxyribonucleoprotein (1 mg/ml). The method used is an indirect immunofluorescent staining method employing FITC-conjugated sheep antihuman immunoglobulin to visualise the antibody. (Magnification x 756)

A negative appearance in the indirect immunofluorescent test for antibody to deoxyribonucleoprotein for comparison with Plate 15. This was obtained using serum from a patient with cryptogenic fibrosing alveolitis (OL.) who had ANA of titre 1 in 100.
(Magnification x 756)

Plate I5

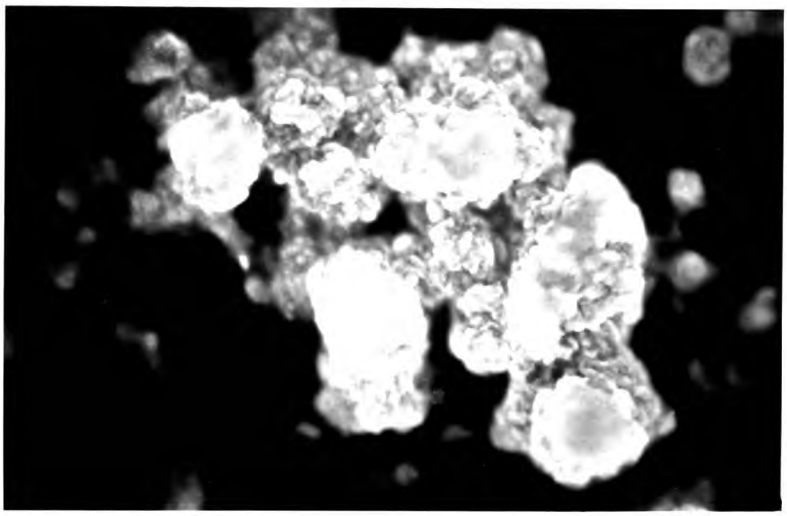
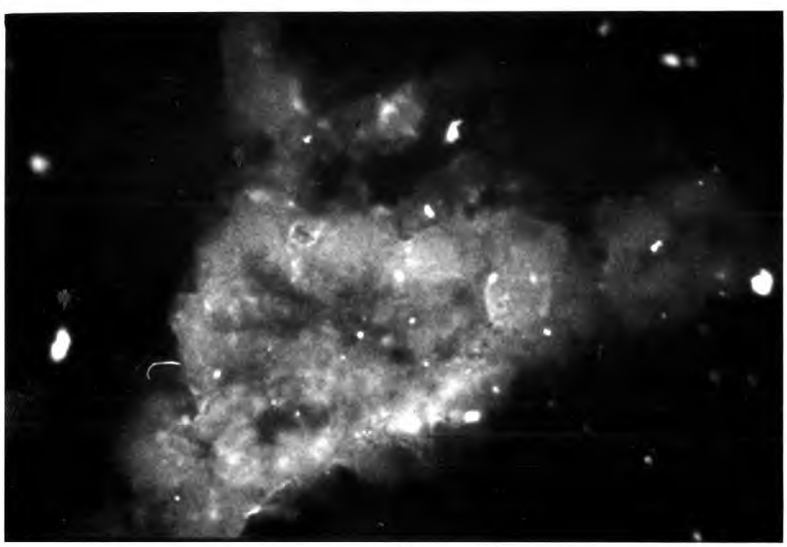


Plate I6



There were a total of 136 ANA positive sera from twenty-eight patients with pleuropulmonary SLE, forty-eight patients with cryptogenic fibrosing alveolitis, twenty-nine patients with asbestosis, twenty-two patients with silicosis and nine patients with intrinsic asthma.

The proportion of the total ANA positive sera with antibodies to deoxyribonucleoprotein in each lung disease group is shown in Table 20 with statistical comparisons in Statistical Table 18, Appendix.

There was a striking disease relationship with a high frequency of deoxyribonucleoprotein antibodies in pleuropulmonary SLE present in 17 (61%) of the 28 ANA positive sera, but in contrast a very low frequency of the antibodies in the other lung diseases. In cryptogenic fibrosing alveolitis they were present in only 6 (12%) of the 48 sera; in intrinsic asthma in 1 (11%) of the 9 sera; in asbestosis in 2 (7%) of the 29 sera and in silicosis in 1 (5%) of the 22 sera.

The statistical analysis (Statistical Table 18) confirmed that the high frequency of deoxyribonucleoprotein antibodies in pleuropulmonary SLE, in contrast with the very low frequency in other lung diseases, was highly significant.

TABLE 20

The proportion of immunofluorescent ANA positive sera containing antibodies reacting with calf thymus deoxyribonucleoprotein.

Disease	Total number of ANA positive sera	Number with antibodies to deoxyribonucleoprotein	
		No.	%
Pleuropulmonary SLE	28	17	61*
CFA	48	6	12
Asbestosis	29	2	7
Silicosis	22	1	5
Intrinsic asthma	9	1	11
Total patients	136	27	20

*Significant increase in comparison with all other diseases (For the detailed statistical analysis, please refer to Appendix , Stat. Table 18).

The Influence of Sex

The results are shown in Table 21 and in Statistical Table 19, Appendix.

There was no relationship between deoxyribonucleoprotein antibodies and sex. The frequency of deoxyribonucleoprotein antibodies in males with pleuropulmonary SLE was high, 57% (4 out of 7 males), and did not differ significantly from the frequency in females 62% (13 out of 21 females). In cryptogenic fibrosing alveolitis the antibodies were present in 19% (5 out of 27) females in comparison with only 5% (1 out of 21) males. However, this difference was not significant.

The frequency of the antibodies in males with pleuropulmonary SLE was significantly higher than in males from all other lung disease groups (Statistical Table 19a). In addition, the frequency in females with pleuropulmonary SLE was significantly above that in females with cryptogenic fibrosing alveolitis ($p < 0.01$) (Statistical Table 19b). An increase over intrinsic asthma could not be confirmed because of the small numbers. The 3 females with asbestosis and the one with silicosis did not have deoxyribonucleoprotein antibodies.

The Influence of Age

There was no relationship between the presence of deoxyribonucleoprotein antibodies and any particular age group.

TABLE 21

A comparison of the proportions of immunofluorescent ANA positive sera with antibodies to calf thymus deoxyribonucleoprotein in the different sexes.

Disease	Sex	Total number of ANA positive sera	Number with antibodies to decxyribonucleoprotein	
			No.	%
Pleuropulmonary SLE	M	7	4	57
	F	21	13	62
CFA	M	21	1	5
	F	27	5	19
Asbestosis	M	26	2	8
	F	3	0	0
Silicosis	M	21	1	5
	F	1	0	0
Intrinsic asthma	M	5	0	0
	F	4	1	25
Total patients	M	80	8	10
	F	56	19	34

CORRELATION BETWEEN DEOXYRIBONUCLEOPROTEIN ANTIBODIES AND LE CELL FORMATION

There was a close correlation between the presence of antibodies to deoxyribonucleoprotein in a patient's serum and the demonstration of LE cell formation.

In pleuropulmonary SLE, LE cell formation had been detected without difficulty on several occasions in 22 out of the total 28 patients, and 16 (73%) out of the 22 had deoxyribonucleoprotein antibodies. There were five other patients in whom LE cell formation had been demonstrated only with difficulty on very few of several occasions when they had been searched for. Just one of these patients had deoxyribonucleoprotein antibodies. There was one other patient with Tart cells instead of the typical LE cell appearance, who also did not have deoxyribonucleoprotein antibodies.

In cryptogenic fibrosing alveolitis, LE cell formation was demonstrated in only 1 of the 48 patients who also had deoxyribonucleoprotein antibodies. Five patients had deoxyribonucleoprotein antibodies but did not have LE cells. The majority, 42, had neither LE cells nor deoxyribonucleoprotein antibodies. (It was interesting that the positive deoxyribonucleoprotein tests were selecting out the rare patients in the cryptogenic fibrosing alveolitis group who had associated liver diseases, either chronic active hepatitis or primary biliary cirrhosis.

In asbestosis, one patient had LE cells, but this was not one of the two with deoxyribonucleoprotein antibodies.

In silicosis, only one patient had LE cells and again this was not the one with deoxyribonucleoprotein antibodies.

In intrinsic asthma, none of the patients had LE cells although one patient had deoxyribonucleoprotein antibodies.

CORRELATION BETWEEN REACTIVITY TO DEOXYRIBONUCLEOPROTEIN AND THE ANA TITRES IN ANA POSITIVE SERA

The results are shown in Table 22 with statistical comparisons.

There was a general relationship between deoxyribonucleoprotein antibodies and high titres of ANA. Overall, the mean log. reciprocal ANA titre for the total 27 sera with deoxyribonucleoprotein antibodies was 2.10 (between 1/80 and 1/160), significantly higher than the mean titre for the total 109 sera without deoxyribonucleoprotein antibodies 1.56 (approximately 1/40); $p < 0.001$.

However, considering the separate disease groups this association only just reached the lowest level of statistical significance. In pleuropulmonary SLE where the majority of the antibodies were found, the mean log. reciprocal ANA titre for the 17 sera with deoxyribonucleoprotein antibodies was 2.25 (between 1/80 and 1/160), compared with a lower mean of 1.82 (between 1/40 and 1/80) for the 11

TABLE 22

A comparison of the immunofluorescent ANA titre distributions in sera with and without antibodies to calf thymus deoxyribonucleoprotein.

Disease	Nucleoprotein antibodies	Total No. of ANA pos. sera	Number and % of total sera at each titre														†Mean ANA titre	S.D.	*Sig. diff. P ≤
			Reciprocal of ANA titre																
			10		20		40		80		160		320		640				
			Logarithm of reciprocal of ANA titre																
			1.0		1.3		1.6		1.9		2.2		2.5		2.8				
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	Present	17	1	6	0	0	2	12	1	6	6	35	2	12	5	29	2.25	0.51	0.1
	Absent	11	3	27	0	0	3	27	0	0	2	18	2	18	1	9	1.82	0.66	
CPA	Present	6	0	0	0	0	1	17	2	33	2	33	1	17	0	0	2.05	0.31	0.1
	Absent	42	11	26	5	12	9	21	10	24	3	7	1	2	3	7	1.63	0.53	
ASB	Present	2	0	0	1	50	1	50	0	0	0	0	0	0	0	0	1.45	0.21	NS
	Absent	27	9	33	9	33	3	11	5	19	0	0	0	0	1	4	1.40	0.43	
SIL	Present	1	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1.30	0.00	
	Absent	21	6	29	7	33	4	19	1	5	2	10	0	0	1	5	1.46	0.48	
AST	Present	1	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1.90	0.00	
	Absent	8	2	25	1	12	1	12	3	38	1	12	0	0	0	0	1.60	0.45	
Total patients	Present	27	1	4	2	7	4	15	4	15	8	30	3	11	5	19	2.10	0.51	0.001
	Absent	109	31	28	22	20	20	18	19	17	8	7	3	3	6	6	1.56	0.51	

*Statistical comparisons using an unpaired Student t test.
†Log. reciprocal.

N.B. % to nearest whole number

sera without antibodies; $p < 0.1$. In cryptogenic fibrosing alveolitis the mean log. reciprocal ANA titre for the 6 sera with deoxyribonucleoprotein antibodies was 2.05 (between 1/80 and 1/160) in contrast with a lower mean 1.63 (1/40) for the 42 sera without antibodies; $p < 0.1$.

In the remaining disease groups, the 2 positive sera in asbestosis had titres of only 1/20 and 1/40; the single positive serum in silicosis had a titre of only 1/20, and the one positive serum in intrinsic asthma had a titre of 1/80.

CORRELATION BETWEEN REACTIVITY TO DEOXYRIBONUCLEOPROTEIN AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA

The results are shown in Table 23 with statistical comparisons.

Deoxyribonucleoprotein antibodies were significantly absent from sera with lone IgM ANA, only 1 (4%) of the total 25 sera with deoxyribonucleoprotein antibodies had lone IgM ANA, in contrast with 15 (19%) of the total 77 sera without such antibodies; $p < 0.1$.

Otherwise the proportions of sera with lone IgG ANA, combined IgG, IgM and IgA ANA's and combined IgG and IgM ANA's were similar in the groups with and without deoxyribonucleoprotein antibodies.

The single patient with IgG and IgA ANA, a patient with pleuropulmonary SLE, did have deoxyribonucleoprotein antibodies, but the one patient with IgM and IgA ANA, in the asbestosis group, did not.

The detailed results for each disease group separately are included in Table 23.

CORRELATION BETWEEN REACTIVITY TO DEOXYRIBONUCLEOPROTEIN AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA

The results are shown in Table 24.

TABLE 23

A comparison of the profiles of immunoglobulin classes of ANA in sera with and without antibodies to calf thymus deoxyribonucleoprotein.

Disease	Nucleoprotein antibodies	Total no. of sera	Immunoglobulin class profile (No. and % of total sera)											
			Lone IgG		Lone IgM		IgG/IgM/IgA		IgG/IgM		IgG/IgA		IgM/IgA	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pleuropulmonary SLE	Present	17	3	18	0	0	8	47	5	29	1	6	0	0
	Absent	10	3	30	1	10	0	0	6	60	0	0	0	0
CFA	Present	4	2	50	0	0	0	0	2	50	0	0	0	0
	Absent	25	7	28	7	28	9	36	2	8	0	0	0	0
Asbestosis	Present	2	1	50	1	50	0	0	0	0	0	0	0	0
	Absent	17	6	35	5	29	2	12	3	18	0	0	1	6
Silicosis	Present	1	1	100	0	0	0	0	0	0	0	0	0	0
	Absent	18	11	61	2	11	3	17	2	11	0	0	0	0
Intrinsic asthma	Present	1	0	0	0	0	0	0	1	100	0	0	0	0
	Absent	7	3	43	0	0	1	14	3	43	0	0	0	0
Total patients	Present	25	7	28	1	4*	8	32	8	32	1	4	0	0
	Absent	77	30	39	15	19	15	19	16	21	0	0	1	1

*Profile significantly less common in sera with deoxyribonucleoprotein antibodies than those without; $p < 0.1$ (Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number.

The speckled pattern of ANA immunofluorescence was rarely seen in sera with deoxyribonucleoprotein antibodies. It was present in only 1 (4%) of the 25 sera with deoxyribonucleoprotein antibodies but in contrast in 12 (16%) of the 77 sera without such antibodies. However, this observation did not achieve statistical significance for such small numbers.

The proportions of sera with homogeneous, peripheral and nucleolar patterns were similar in the groups with and without antibodies to deoxyribonucleoprotein.

CORRELATION BETWEEN REACTIVITY TO DEOXYRIBONUCLEOPROTEIN AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA

The results are shown in Table 25.

There was a general relationship indicating that deoxyribonucleoprotein antibodies tended to occur in the same disease groups as those with complement fixing ANA, but within these disease groups it was seen that there was no significant association between the two.

Complement fixing ANA was present in 10 (38%) of the total 26 sera with deoxyribonucleoprotein antibodies, in comparison with 20 (20%) of the total 99 sera without antibodies; $p < 0.1$.

For the separate disease groups: in pleuropulmonary SLE, complement fixing ANA was present in 6 (35%) out of 17 sera with deoxyribonucleo-

TABLE 24

A comparison of the patterns of ANA immunofluorescence in sera with and without antibodies to calf thymus deoxyribonucleoprotein.

Patterns	Number of sera with Nucleoprotein antibodies							Number of sera without Nucleoprotein antibodies						
	Total sera		SLE	CFA	ASB	SIL	AST	Total sera		SLE	CFA	ASB	SIL	AST
	25	(100%)	16	4	2	1	1	77	(100%)	10	25	17	18	7
Homogeneous pattern	22	88	15	4	1	1	1	60	77	8	18	16	13	5
Lone pattern	15	60	10	3	1	1	0	48	62	7	12	14	12	3
In combination	7	28	5	1	0	0	1	12	16	1	6	2	1	2
Peripheral pattern	4	16	3	0	0	0	1	19	25	0	6	5	5	3
Lone pattern	1	4	1	0	0	0	0	13	17	0	4	3	4	2
In combination	3	12	2	0	0	0	1	6	8	0	2	2	1	1
Speckled pattern	1	4	1	0	0	0	0	12	16	6	3	2	0	1
Lone pattern	0	0	0	0	0	0	0	5	6	1	2	2	0	0
In combination	1	4	1	0	0	0	0	7	9	5	1	0	0	1
Nucleolar pattern	4	16	3	1	0	0	0	10	13	2	7	0	1	0
Lone pattern	0	4	0	1	0	0	0	2	3	0	1	0	1	0
In combination	3	12	3	0	0	0	0	8	10	2	6	0	0	0

Statistical comparisons, using a test of difference of proportions, showed no significant differences.

N.B. % to nearest whole number

TABLE 25

The extent of association between ANAs reacting with calf thymus deoxyribonucleoprotein and those fixing complement (in immunofluorescent ANA positive sera).

Disease	Nucleoprotein antibodies	Complement fixing ANA (No. of sera) Present	Absent	Totals	*Significant association $p \leq$
Pleuropulmonary SLE	Present	6	11	17	
	Absent	5	6	11	NS
	Totals	11	17	28	
CFA	Present	4	2	6	
	Absent	12	26	38	NS
	Totals	16	28	44	
Asbestosis	Present	0	2	2	
	Absent	0	21	21	
	Totals	0	23	23	
Silicosis	Present	0	1	1	
	Absent	3	18	21	
	Totals	3	19	22	
Intrinsic asthma	Present	0	1	1	
	Absent	0	8	8	
	Totals	0	9	9	
Total patients	Present	10	16	26	
	Absent	20	79	99	0.1
	Totals	30	95	125	

*Statistical analysis using a Fourfold table test for groups < 50 and a Chi square test for groups ≥ 50.

protein antibodies, and also in 5 (45%) of the 11 sera without such antibodies. In cryptogenic fibrosing alveolitis, complement fixing ANA was present in 4 (67%) of the 6 sera with deoxyribonucleoprotein antibodies, and in 12 (32%) of the 38 sera without the antibodies. In the remaining lung diseases, none of the 3 sera with deoxyribonucleoprotein antibodies had complement fixing ANA.

VII. ANTIBODIES PRECIPITATING WITH CALF THYMUS 'DOUBLE STRANDED' DEOXYRIBONUCLEIC ACID ('DS'-DNA)

RESULT OF AN ATTEMPT TO REDUCE SINGLE STRANDED (SS) DNA CONTAMINATION FROM COMMERCIAL CALF THYMUS 'DS'-DNA BY TREATMENT WITH NUCLEASE SI

The results of an attempt to determine the practical value of using Nuclease SI to remove SS-DNA from damaged commercial calf thymus 'DS'-DNA (p. 77) are shown in Table 26 a and b.

Parallel Ouchterlony double diffusion immunoprecipitation tests on the 28 ANA positive sera from patients with pleuropulmonary SLE comparing 'impure' and Nuclease S1 'purified' commercial calf thymus DNA antigens, showed precipitins in 16 sera using 'impure' DNA, and in 10 of these using the 'purified' DNA (Table 26 a and b).

Since 6 sera reacted only with the 'impure' DNA (Table 26b) it was concluded that these probably had precipitins to SS-DNA and that the enzyme treatment had at least achieved sufficient reduction in SS-DNA contamination to negate the reactions in these 6 instances.

Examples of these results are shown in Plate 17 with 'impure' DNA as the antigen in the central well and undiluted precipitin positive and

TABLE 26

Trial to determine the practical value of Nuclease S1 treatment to reduce SS-DNA contamination in commercial calf thymus DNA.

a. A comparison of the numbers of SLE sera positive in Ouchterlony precipitation tests using untreated ('impure'), and Nuclease S1-treated ('purified') commercial calf thymus DNA

	Total sera	No. with pos. precipitins using: 'Impure' DNA		'Purified DNA'	
		No.	%	No.	%
Pleuropulmonary SLE	28	16	57	10	36

b. The correlation of the Ouchterlony test results using 'impure' and those using 'purified' DNA

	Total sera	No. of sera reacting with: 'Impure' DNA only	'Purified' DNA only	Both	Neither
Pleuropulmonary SLE	28	6	0	10	12

Ouchterlony gel double-diffusion test using untreated ('impure') commercial calf thymus DNA as the antigen in the central well and undiluted sera from different patients with SLE in the eight peripheral wells. The sera in wells 2, 3, 5 and 8 show lines of positive precipitation with DNA. (Magnification x 4)

Ouchterlony gel double-diffusion test conducted simultaneously with that in Plate 17 with the same eight SLE sera in the peripheral wells but using Nuclease S1 'purified' commercial calf thymus DNA (1 mg/ml) in the central well. The serum in well 5 positive with 'impure' DNA, is clearly negative with the 'purified' DNA. (Magnification x 4)

Plate 17

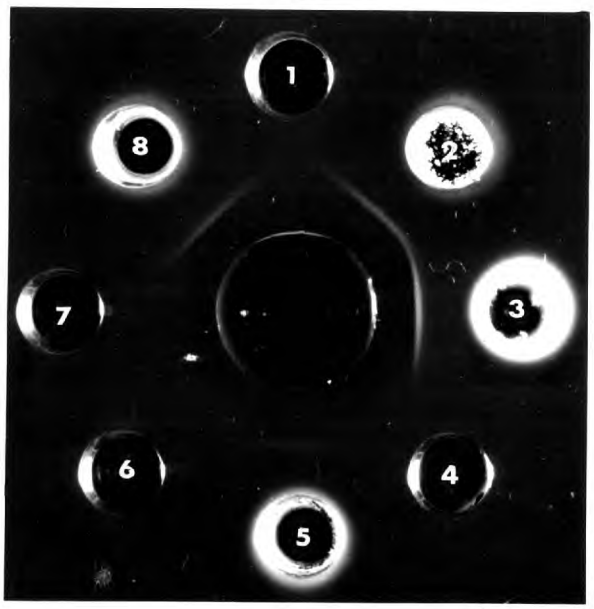
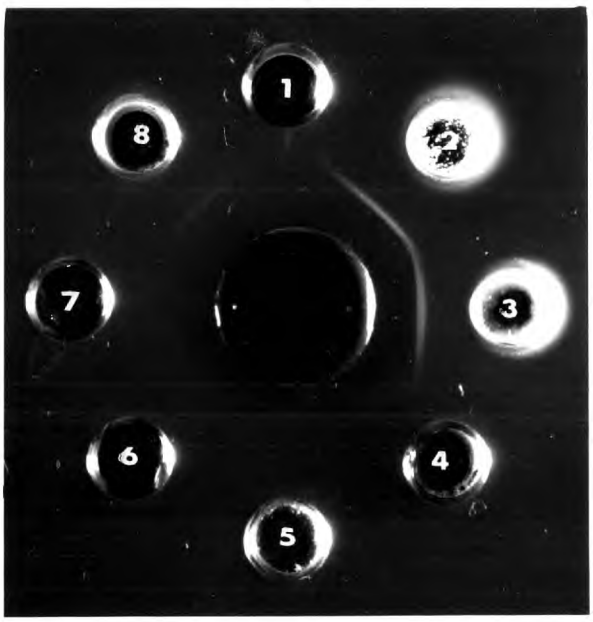


Plate 18



negative sera in the outer wells, and in Plate 18 which shows the exact replica but with 'purified' DNA as the antigen. It can be seen that serum 5 positive with 'impure' DNA is negative with 'purified' DNA.

The conclusion was to use Nuclease S1 'purified' calf thymus 'DS'-DNA as the antigen to test sera from the remaining lung diseases in the absence of a proven double stranded DNA, but allowing that although improved over the untreated commercial DNA it might still contain some SS-DNA.

NORMAL CONTROL STUDIES

Only one (a male) of the ten ANA positive sera (5 males and 5 females) from normal controls without lung disease had an Ouchterlony precipitin line to Nuclease Si-treated calf thymus 'DS'-DNA.

LUNG DISEASE STUDIES

The ANA positive sera used for this investigation were the same as those used to investigate ANA titres, immunoglobulin classes of ANA, patterns of ANA immunofluorescence, complement fixing ability of ANA and deoxyribonucleoprotein antibodies. The differences in numbers related to insufficient serum in some instances.

There were a total of 142 ANA positive sera from 28 patients with pleuropulmonary SLE; 52 patients with cryptogenic fibrosing alveolitis; 31 patients with asbestosis; 22 patients with silicosis and 9 patients with intrinsic asthma.

The proportion of the total ANA positive sera with Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA in each lung disease group is shown in Table 27 with statistical comparisons in Statistical Table 20, Appendix.

Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA were present in 50 (35%) of the total 142 ANA positive sera.

They were present in all lung disease groups except intrinsic asthma, and there was a higher frequency in silicosis, 59% (13 out of 22 sera), than in any other lung disease. In cryptogenic fibrosing alveolitis they were seen in 40% (21 out of 52) of sera, and in pleuropulmonary SLE in 36% (10 out of 28) of sera. They were less common in asbestosis, in only 19% (6 out of 31) of sera, and were not seen at all in intrinsic asthma (0 out of 9 sera).

The statistical analysis (Statistical Table 20) confirmed that the frequency of Nuclease SI-treated calf thymus 'DS'-DNA Ouchterlony precipitins was significantly higher in silicosis than in any other lung disease except cryptogenic fibrosing alveolitis.

TABLE 27

The proportion of immunofluorescent ANA positive sera containing Ouchterlony precipitins to Nuclease S1-treated calf thymus 'DS'-DNA

Disease	Total number of ANA positive sera	Number with precipitins to Nuclease S1-treated 'DS' - DNA	
		No.	%
Pleuropulmonary SLE	28	10	36
CFA	52	21	40
Asbestosis	31	6	19
Silicosis	22	13	59
Intrinsic asthma	9	0	0
Total patients	142	50	35

In addition it was confirmed that the frequency of these precipitins was significantly lower in asbestosis and intrinsic asthma than in the other lung diseases, although asbestosis could not be shown to differ significantly from pleuropulmonary SLE.

The Influence of Sex

The results are shown in Table 28.

There was a tendency for females to have a slightly higher frequency of Nuclease SI-treated calf thymus 'DS'-DNA Ouchterlony precipitins than males, although this was not significant in the statistical tests.

The higher frequency of Nuclease SI-treated calf thymus 'DS'-DNA precipitins in silicosis than in the other lung diseases was still significant when sex was taken into account (Statistical Table 21, Appendix). Silicosis was mainly a male group and precipitins were present in 57% (12 out of 21) of the male patients, more than in males with cryptogenic fibrosing alveolitis 33% (8 out of 24 males), and significantly more than in males with asbestosis 18% (5 out of 28 males), males with pleuropulmonary SLE 14% (1 out of 7 males) or males with intrinsic asthma (0 out of 5 males).

The Influence of Age

There was no relationship between the presence of Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA and any particular age range.

TABLE 28

A comparison of the proportion of immunofluorescent ANA positive sera with Ouchterlony precipitins to Nuclease S1-treated calf thymus 'DS' - DNA in the different sexes.

Disease	Sex	Total number of ANA positive sera	Number with precipitins to Nuclease S1-treated 'DS' - DNA	
			No.	%*
Pleuropulmonary SLE	M	7	1	14
	F	21	9	43
CFA	M	24	8	33
	F	28	13	46
Asbestosis	M	28	5	18
	F	3	1	33
Silicosis	M	21	12	57
	F	1	1	100
Intrinsic asthma	M	5	0	0
	F	4	0	0
Total patients	M	85	26	30
	F	57	24	42

*Differences between males and females were not statistically significant using a test of difference of proportions.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE ANA TITRES IN ANA POSITIVE SERA

The results are shown in Table 29.

There was no significant relationship between the presence of Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA precipitins and any particular range of ANA titres. The range of ANA titres in sera with, and without precipitins was similarly wide involving both higher and lower titres of ANA. The mean log. reciprocal ANA titre for the total 50 sera with precipitins was 1.65 (approximately 1/40) not significantly different from the mean 1.58 (between 1/20 and 1/40) for the total 91 sera without precipitins.

The detailed results within each separate lung disease group can be obtained by reference to the Table 29.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA

There was no general relationship between the presence of Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA and any particular profile of immunoglobulin classes of ANA. The proportions

TABLE 29

A comparison of the immunofluorescent ANA titre distributions in sera with and without precipitins to Nuclease S1-treated 'DS'-DNA.

Disease	'DS'-DNA precipitins	Total No. of ANA pos. sera	Number and % of total sera at each titre														/Mean ANA titre	S.D.	*Sig. diff. p >
			Reciprocal of ANA titre																
			10		20		40		80		160		320		640				
			Logarithm of reciprocal of ANA titre																
			1.0		1.3		1.6		1.9		2.2		2.5		2.8				
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	Present	10	2	20	0	0	1	10	0	0	3	30	2	20	2	20	2.08	0.67	NS
	Absent	17	2	12	0	0	4	24	1	6	4	24	2	12	4	24	2.08	0.60	
CFA	Present	21	6	29	2	10	1	5	6	29	5	24	1	5	0	0	1.67	0.52	NS
	Absent	31	11	35	3	10	9	29	5	16	0	0	0	0	3	10	1.52	0.54	
ASB	Present	6	3	50	2	33	1	17	0	0	0	0	0	0	0	0	1.20	0.24	NS
	Absent	25	6	24	9	36	4	16	5	20	0	0	0	0	1	4	1.46	0.43	
SIL	Present	13	2	15	6	46	3	23	0	0	1	8	0	0	1	8	1.51	0.50	NS
	Absent	9	4	44	2	22	1	11	1	11	1	11	0	0	0	0	1.37	0.44	
AST	Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
	Absent	9	2	22	1	11	1	11	4	44	1	11	0	0	0	0	1.63	0.44	
Total patients	Present	50	13	26	10	20	6	12	6	12	9	18	3	6	3	6	1.65	0.57	NS
	Absent	91	25	27	15	16	19	21	16	17	6	7	2	2	8	9	1.58	0.58	

*Statistical comparisons using an unpaired Student t test.

/Log. reciprocal.

N.B. % to nearest whole number

of sera with the various immunoglobulin profiles were similar in the groups with, and without precipitins, and no significant differences were observed in a statistical analysis.

The detailed results including those for each lung disease separately are shown in Table 30.

It was noted that while in cryptogenic fibrosing alveolitis, asbestosis and silicosis the majority of sera with precipitins had lone IgG ANA, in contrast in pleuropulmonary SLE the majority had either IgG, IgM and IgA ANA's (4 (44%) out of 9 sera) or IgG and IgM ANA's (4 (44%) out of 9 sera), and only one serum had lone IgG ANA. It was not possible to confirm the reliability of this observation for such small numbers.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND THE PATTERNS OF ANA IMMUNO-FLUORESCENCE IN ANA POSITIVE SERA

The results are shown in Table 31 with statistical comparisons.

There was a significant relationship between the speckled and nucleolar immunofluorescent staining patterns and the presence of Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA. These patterns were rarely seen in sera without such precipitins.

TABLE 30

A comparison of the profiles of immunoglobulin classes of ANA in sera with and without precipitins to Nuclease S1-treated 'DS'-DNA.

Disease	'DS'-DNA precipitins	Total no. of sera	Immunoglobulin class profile (No. and % of total sera)											
			Lone IgG		Lone IgM		IgG/IgM/IgA		IgG/IgM		IgG/IgA		IgM/IgA	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pleuropulmonary SLE	Present	9	1	11	0	0	4	44	4	44	0	0	0	0
	Absent	18	5	28	1	6	4	22	7	39	1	6	0	0
CFA	Present	14	6	43	2	14	4	29	2	14	0	0	0	0
	Absent	16	3	19	5	31	5	31	3	19	0	0	0	0
Asbestosis	Present	5	3	60	1	20	0	0	1	20	0	0	0	0
	Absent	16	3	19	7	44	2	12	3	19	0	0	1	6
Silicosis	Present	12	6	50	2	17	2	17	2	17	0	0	0	0
	Absent	7	6	86	0	0	1	14	0	0	0	0	0	0
Intrinsic asthma	Present	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	8	3	38	0	0	1	12	4	50	0	0	0	0
Total patients	Present	40	16	40	5	12	10	25	9	23	0	0	0	0
	Absent	65	20	31	13	20	13	20	17	26	1	1	1	1

(Statistical comparisons, using a test of difference of proportions, showed no significant differences).

N.B. % to nearest whole number.

TABLE 31

A comparison of the patterns of ANA immunofluorescence in sera with and without Ouchterlony precipitins to Nuclease S1-treated calf thymus 'DS'-DNA.

Patterns	Number of sera with 'DS'-DNA precipitins							Number of sera without 'DS'-DNA precipitins						
	Total sera		SLE	CFA	ASB	SIL	AST	Total sera		SLE	CFA	ASB	SIL	AST
	40	(100%)	9	14	5	12	0	65	(100%)	18	16	16	7	8
Homogeneous pattern	29	73	7	9	4	9	0	53	81	16	13	13	5	6
Lone pattern	21	53	2	7	4	8	0	42	64	15	8	11	5	3
In combination	8	20	5	2	0	1	0	11	17	1	5	2	0	3
Peripheral pattern	6	15	1	2	0	3	0	17	26	2	4	5	2	4
Lone pattern	3	8	0	1	0	2	0	11	17	1	3	3	2	2
In combination	3	8	1	1	0	1	0	6	9	1	1	2	0	2
Speckled pattern	9	23*	5	3	1	0	0	3	5	1	0	1	0	1
Lone pattern	3	8	0	2	1	0	0	2	3	1	0	1	0	0
In combination	6	15	5	1	0	0	0	1	2	0	0	0	0	1
Nucleolar pattern	8	20*	4	3	0	1	0	5	8	1	4	0	0	0
Lone pattern	1	3	0	1	0	1	0	0	0	0	0	0	0	0
In combination	7	18	4	3	0	0	0	5	8	1	4	0	0	0

*Speckled and nucleolar patterns significantly more common in sera with precipitins than those without; $p < 0.01$ and $p < 0.1$ respectively.
(Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number

The speckled pattern was seen in 9 (23%) of a total of 40 sera with precipitins, compared with only 3 (5%) of a total 65 sera without precipitins; $p < 0.01$. The nucleolar pattern was seen in 8 (20%) of the total 40 sera with precipitins, compared with only 5 (8%) of the total 65 sera without precipitins; $p < 0.01$.

Similar proportions of sera with homogeneous and peripheral patterns were observed in the groups with and without precipitins. The homogeneous pattern was the most frequent pattern in both groups, and was seen in 29 (73%) of the 40 sera with precipitins, and in 53 (81%) of the 65 sera without precipitins. In both situations the pattern was more frequently the lone pattern in the serum, than one of a combination of different patterns. The peripheral pattern was present in 6 (15%) of the 40 sera with precipitins, and in 17 (26%) of the 65 sera without precipitins.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA

The results are shown in Table 32.

There was a significant general relationship between Ouchterlony precipitation with Nuclease SI-treated calf thymus 'DS'-DNA and

TABLE 32

The extent of association between ANAs precipitating with Nuclease S1-treated calf thymus 'DS'-DNA and those fixing complement (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA precipitins	Complement fixing ANA (No. of sera)			*Significant association
		Present	Absent	Totals	$P \leqslant$
Pleuropulmonary SLE	Present	6	4	10	
	Absent	5	13	18	NS
	Totals	11	17	28	
CFA	Present	10	9	19	
	Absent	7	21	28	NS
	Totals	17	30	47	
Asbestosis	Present	0	6	6	
	Absent	0	18	18	
	Totals	0	24	24	
Silicosis	Present	2	11	13	
	Absent	1	8	9	
	Totals	3	19	22	
Intrinsic asthma	Present	0	0	0	
	Absent	0	9	9	
	Totals	0	9	9	
Total patients	Present	18	30	48	
	Absent	13	69	82	0.01
	Totals	31	99	130	

*Statistical analysis using a Fourfold table test for groups < 50 and a Chi square test for groups $\geqslant 50$.

complement fixing ability in ANA positive sera. Eighteen (37%) of the total 48 sera with precipitins also had complement fixing ANA, in comparison with only 13 (16%) of the total 82 sera without precipitins; $p < 0.01$.)

However, the relationship was not confirmed in the separate disease groups. In cryptogenic fibrosing alveolitis 10 (53%) out of 19 sera with precipitins had complement fixing ANA, in comparison with 7 (25%) out of 28 sera without precipitins. In pleuropulmonary SLE 6 (60%) of the 10 sera with precipitins had complement fixing ANA, in comparison with 5 (28%) of 18 sera without precipitins. In asbestosis there were no sera with complement fixing ANA, but 6 had precipitins. In silicosis 2 (15%) of 13 sera with precipitins had complement fixing ANA and 1 (11%) of 9 sera without precipitins.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA AND REACTIVITY TO CALF THYMUS DEOXYRIBONUCLEOPROTEIN IN ANA POSITIVE SERA

The results are shown in Table 33.

There was no significant relationship between Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA and deoxyribonucleoprotein antibodies. Overall, antibodies to deoxyribonucleoprotein

TABLE 33

The extent of association between ANAs precipiting with Nuclease S1-treated 'DS'-DNA and those reacting with calf thymus deoxyribonucleoprotein (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA precipitins	Nucleoprotein antibodies (No. of sera) Present	Absent	Totals	*Significant association $p \leq$
Pleuropulmonary SLE	Present	6	4	10	
	Absent	11	7	18	NS
	Totals	17	11	28	
CFA	Present	4	13	17	
	Absent	2	25	47	NS
	Totals	6	38	64	
Asbestosis	Present	0	4	4	
	Absent	1	23	24	
	Totals	1	27	28	
Silicosis	Present	1	12	13	
	Absent	0	9	9	
	Totals	1	21	22	
Intrinsic asthma	Present	0	0	0	
	Absent	1	8	9	
	Totals	1	8	9	
Total patients	Present	11	33	44	
	Absent	15	72	87	NS
	Totals	26	105	131	

*Statistical analysis using a Fourfold table test for groups < 50 and a Chi square test for groups ≥ 50.

were present in 11 (25%) of a total 44 sera with precipitins, and in 15 (17%) of a total of 87 sera without precipitins. The difference was not significant.

The detailed results for each lung disease group (Table 33) also confirmed the absence of any significant association between the two types of antibody.

VIII. ANTIBODIES PRECIPITATING WITH CALF THYMUS SINGLE STRANDED DEOXYRIBONUCLEIC ACID (SS-DNA)

The precipitin lines observed when calf thymus SS-DNA was used as the antigen in Ouchterlony double diffusion immunoprecipitation tests were generally heavy and there were very few doubtful lines of weak precipitation. Examples are shown in Plate 19.

Plate 20, is a replica except that calf thymus 'DS'-DNA was the specific antigen in the central well.

Both tests were set up at the same time and under the same conditions. Direct comparison of the two Plates illustrates that the precipitation lines observed with SS-DNA were generally heavier than those observed with 'DS'-DNA in those sera reacting with both SS and 'DS'-DNA.

NORMAL CONTROL STUDIES

Ouchterlony precipitins to calf thymus SS-DNA were seen in only one (a female) of the ten ANA positive sera (5 males and 5 females) from normal controls without lung disease.

LUNG DISEASE STUDIES

The ANA positive sera used for this investigation were the same as

Ouchterlony gel double-diffusion showing 'heavy' lines of precipitation obtained with undiluted sera from three patients with SLE in peripheral wells 1, 2 and 3 and heat-denatured (single-stranded) calf thymus DNA (1 mg/ml) in the central well. Peripheral well 4 contains a precipitin negative serum from a patient with SLE. (Magnification x 4)

Ouchterlony gel double-diffusion showing 'weaker' lines of precipitation by comparison with the appearancesin Plate 19 using the same SLE sera in the four peripheral wells but the unheated 'double-stranded' calf thymus DNA (1 mg/ml) in the central well. (Magnification x 4)

Plate I9

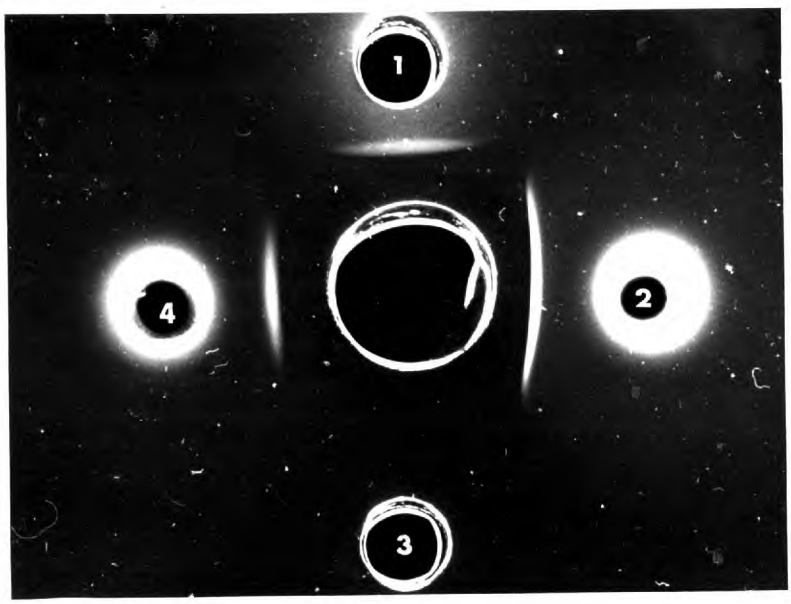
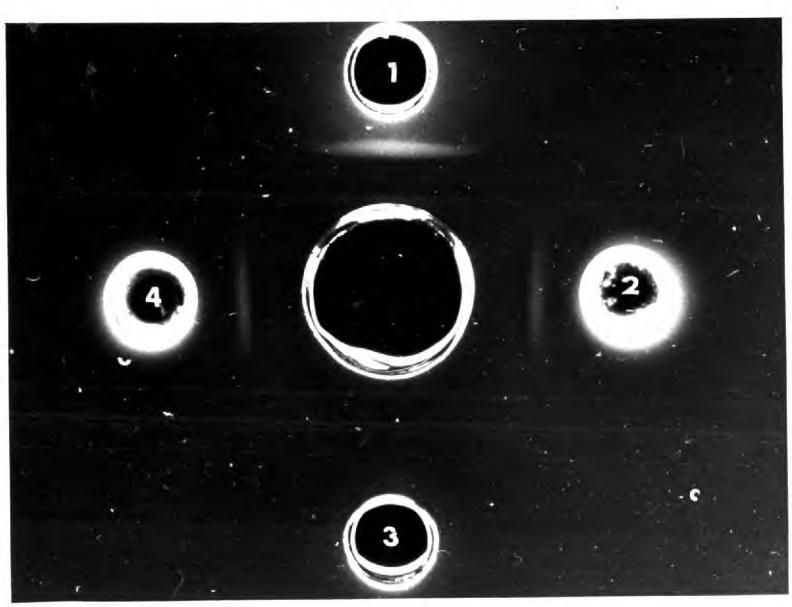


Plate 20



those used to investigate ANA titres, immunoglobulin classes of ANA, patterns of ANA immunofluorescence, complement fixing ability of ANA, deoxyribonucleoprotein antibodies and 'DS'-DNA precipitins. The differences in numbers related to insufficient serum in some instances.

There were a total of 138 ANA positive sera from 28 patients with pleuropulmonary SLE, 52 patients with cryptogenic fibrosing alveolitis, 27 patients with asbestosis, 22 patients with silicosis and 9 patients with intrinsic asthma.

The proportion of the total ANA positive sera with Ouchterlony precipitins to calf thymus SS-DNA in each lung disease group is shown in Table 34 with statistical comparisons in Statistical Table 22, Appendix.

Ouchterlony precipitins to calf thymus SS-DNA were seen in 51 (37%) of the total 138 ANA positive sera from patients with lung disease.

The disease with the highest frequency of calf thymus SS-DNA Ouchterlony precipitins was pleuropulmonary SLE, in 57% (16 out of 28) of sera. The frequency in silicosis was 41% (9 out of 22 sera) and in cryptogenic fibrosing alveolitis 36% (19 out of 52 sera), and was lowest in asbestosis 22% (6 out of 27 sera) and in intrinsic asthma 11% (1 out of 9 sera).

The statistical analysis (Statistical Table 22) confirmed that pleuropulmonary SLE had a significantly higher frequency of calf thymus

TABLE 34

The proportion of immunofluorescent ANA positive sera with Ouchterlony precipitins to calf thymus SS-DNA

Disease	Total number of ANA positive sera	Number with precipitins to SS - DNA	
		No.	%
Pleuropulmonary SLE	28	16	57*
CFA	52	19	36
Asbestosis	27	6	22
Silicosis	22	9	41
Intrinsic asthma	9	1	11
Total patients	138	51	37

*Significantly higher frequency than in all other diseases except silicosis.
(For the detailed statistical analysis, please refer to Appendix, Stat. Table 22).

SS-DNA Ouchterlony precipitins than any other lung disease except silicosis. However, the frequencies of the precipitins in the remaining lung diseases including silicosis, could not be shown to differ significantly.

The Influence of Sex

The results are shown in Table 35 with statistical comparisons in Statistical Table 23, Appendix.

There was a significant trend towards a higher frequency of calf thymus SS-DNA Ouchterlony precipitins in females than in males. In pleuropulmonary SLE the proportion of calf thymus SS-DNA Ouchterlony precipitins in female patients was 67% (14 out of 21) compared with only 29% (2 out of 7) in the males; $p < 0.1$. In cryptogenic fibrosing alveolitis, the precipitins were present in 50% (14 out of 28) of the females, compared with only 21% (5 out of 24) of the males; $p < 0.05$.

The previous observation of a significantly higher proportion of calf thymus SS-DNA Ouchterlony precipitins in pleuropulmonary SLE than in the other lung diseases except silicosis could not be confirmed and was apparently a reflection of sex not disease differences. The proportion of the precipitins in males with pleuropulmonary SLE did not differ significantly from that in males with any other lung disease. In addition the proportion in females with pleuropulmonary SLE was not significantly different from that in females with cryptogenic fibrosing alveolitis. The intrinsic

TABLE 35

A comparison of the proportions of immunofluorescent ANA positive sera with Ouchterlony precipitins to calf thymus SS-DNA in the different sexes.

Disease	Sex	Total number of ANA positive sera	Number with precipitins to SS - DNA	
			No.	%
Pleuropulmonary SLE	M	7	2	29
	F	21	14	67*
CFA	M	24	5	21
	F	28	14	50*
Asbestosis	M	24	5	21
	F	3	1	33
Silicosis	M	21	8	41
	F	1	1	100
Intrinsic asthma	M	5	0	0
	F	4	1	25
Total patients	M	81	20	25
	F	57	31	54*

*Significantly higher frequency in females than in males.
(For the detailed statistical analysis, please refer to Appendix , Stat. Table 23).

asthma group was too small for reliable statistical analysis.

It was noted that males with silicosis did have a higher frequency of calf thymus SS-DNA Ouchterlony precipitins, 41% (8 out of 21 males), than males in any other lung disease group, although this was not confirmed by statistical tests.

The Influence of Age

There was no relationship between the presence of Ouchterlony precipitins to calf thymus SS-DNA and any particular age range.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE ANA TITRES IN ANA POSITIVE SERA

The results are shown in Table 36.

There was a tendency for the range of ANA titres to be slightly higher in sera with calf thymus SS-DNA precipitins, than in sera without precipitins. Overall, the mean log. reciprocal ANA titre for the total 51 sera with calf thymus SS-DNA precipitins was 1.80 (between 1 in 40 and 1 in 80), significantly higher than that for the total 87 sera without precipitins, 1.53 (between 1 in 20 and 1 in 40); $p < 0.01$.

TABLE 36

A comparison of the immunofluorescent ANA titre distributions in sera with and without precipitins to calf thymus SS-DNA.

Disease	SS-DNA precipitins	Total No. of ANA pos. sera	Number and % of total sera at each titre														$\neq$ Mean ANA titre	S.D.	*Sig. diff. P $\leq$
			Reciprocal of ANA titre																
			10		20		40		80		160		320		640				
			Logarithm of reciprocal of ANA titre																
			1.0		1.3		1.6		1.9		2.2		2.5		2.8				
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	Present	16	1	6	0	0	3	19	0	0	6	38	2	12	4	25	2.20	0.53	NS
	Absent	12	3	25	0	0	2	17	1	8	2	17	2	17	2	17	1.93	0.68	
CFA	Present	19	4	21	3	16	2	11	5	26	4	21	1	5	0	0	1.68	0.49	NS
	Absent	33	13	39	2	6	8	24	6	18	1	3	0	0	3	9	1.53	0.56	
ASB	Present	6	3	50	1	17	1	17	1	17	0	0	0	0	0	0	1.30	0.38	NS
	Absent	21	6	29	7	33	3	14	4	19	0	0	0	0	1	5	1.44	0.45	
SIL	Present	9	1	11	3	33	2	22	1	11	1	11	0	0	1	11	1.67	0.56	0.1
	Absent	13	5	38	5	38	2	15	0	0	1	8	0	0	0	0	1.30	0.35	
AST	Present	1	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1.90	0	
	Absent	8	2	25	1	12	1	12	3	38	1	12	0	0	0	0	1.60	0.45	
Total patients	Present	51	9	18	7	14	8	16	8	16	11	22	3	6	5	10	1.80	0.57	0.01
	Absent	87	29	33	15	17	16	18	14	16	5	6	2	2	6	7	1.53	0.54	

*Statistical comparisons using an unpaired Student t test.
$\neq$ Log. reciprocal.

N.B. % to nearest whole number.

However, in the separate disease groups, although the mean ANA titres were higher in sera with precipitins than in those without precipitins, the trend was significant only in silicosis where the mean log. reciprocal ANA titre for the 9 sera with precipitins was 1.67 (approximately 1 in 40) in contrast with 1.30 (1 in 20) for the 13 sera without precipitins; $p < 0.1$.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA

The results are shown in Table 37.

Ouchterlony precipitins to calf thymus SS-DNA were rarely seen in sera with lone IgM ANA. Only 2 (4%) of the total 44 sera with precipitins had lone IgM ANA, significantly less than for sera without precipitins where 14 (24%) of the total 58 sera had lone IgM ANA; $p < 0.01$.

The IgG, IgM and IgA ANA combination was slightly more common in sera with precipitins than in those without precipitins, but the difference was not significant. Overall the combination was seen in 13 (30%) of the total 44 sera with precipitins compared with 10 (17%) of the total 58 sera without precipitins.

The remaining immunoglobulin profiles were seen similarly in sera with and without precipitins. (The detailed results are shown in Table 37).

TABLE 37

A comparison of the profiles of immunoglobulin classes of ANA in sera with and without precipitins to calf thymus SS-DNA

Disease	SS-DNA precipitins	Total no. of sera	Immunoglobulin class profile (No. and % of total sera)											
			Lone IgG		Lone IgM		IgG/IgM/IgA		IgG/IgM		IgG/IgA		IgM/IgA	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pleuropulmonary SLE	Present	16	4	25	0	0	6	37	6	37	0	0	0	0
	Absent	11	2	18	1	9	2	18	5	46	1	9	0	0
CFA	Present	12	4	33	0	0	4	33	4	33	0	0	0	0
	Absent	18	5	28	7	39	5	28	1	6	0	0	0	0
Asbestosis	Present	6	4	67	1	17	1	17	0	0	0	0	0	0
	Absent	12	2	17	5	42	1	8	3	25	0	0	1	8
Silicosis	Present	9	5	56	1	11	2	22	1	11	0	0	0	0
	Absent	10	7	70	1	10	1	10	1	10	0	0	0	0
Intrinsic asthma	Present	1	0	0	0	0	0	0	1	100	0	0	0	0
	Absent	7	3	43	0	0	1	14	3	43	0	0	0	0
Total patients	Present	44	17	39	2	4*	13	30	12	27	0	0	0	0
	Absent	58	19	33	14	24	10	17	13	22	1	2	1	2

*Profile significantly less common in sera with SS-DNA precipitins than those without; $p < 0.01$. (Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA

The results are shown in Table 38.

There was a significant association between the speckled immunofluorescent staining pattern and Ouchterlony precipitins to calf thymus SS-DNA. It was seen in 9 (20%) of the total 44 sera with precipitins, compared with only 3 (5%) of the total 58 sera without precipitins; $p < 0.02$.

The homogeneous, peripheral and nucleolar patterns were seen in sera with and without precipitins and the proportions did not differ significantly. The homogeneous pattern was present in 34 (77%) of the total 44 sera with precipitins, and similarly in 44 (76%) of the 58 sera without precipitins. There was a lower frequency of the peripheral pattern in sera with precipitins, in 7 (16%) of the 44 sera, than in sera without precipitins, in 16 (28%) of the 58 sera, but this difference was not significant. The nucleolar pattern was seen in a higher proportion of sera with precipitins, 8 (18%) of the 44 sera, than in sera without precipitins, 5 (9%) of the 58 sera, but again this difference was not significant.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA

The results are shown in Table 39 with statistical comparisons.

TABLE 38

A comparison of the patterns of ANA immunofluorescence in sera with and without Ouchterlony precipitins to calf thymus SS-DNA.

Patterns	Number of sera with SS-DNA precipitins							Number of sera without SS-DNA precipitins						
	Total sera		SLE	CFA	ASB	SIL	AST	Total sera		SLE	CFA	ASB	SIL	AST
	44	(100%)	16	12	6	9	1	58	(100%)	11	18	12	10	7
Homogeneous pattern	34	77	12	9	5	7	1	44	76	11	13	9	6	5
Lone pattern	24	55	7	6	4	7	0	36	62	10	9	8	6	3
In combination	10	23	5	3	1	0	1	8	14	1	4	1	0	2
Peripheral pattern	7	16	2	1	2	1	1	16	28	1	5	3	4	3
Lone pattern	3	7	1	0	1	1	0	11	19	0	4	2	3	2
In combination	4	9	1	1	1	0	1	5	9	1	1	1	1	1
Speckled pattern	9	20*	6	3	0	0	0	3	5	0	0	2	0	1
Lone pattern	3	7	1	2	0	0	0	2	3	0	0	2	0	0
In combination	6	14	5	1	0	0	0	1	2	0	0	0	0	1
Nucleolar pattern	8	18	4	3	0	1	0	5	9	1	4	0	0	0
Lone pattern	1	2	0	0	0	1	0	1	2	0	1	0	0	0
In combination	7	16	4	3	0	0	0	4	7	1	3	0	0	0

*Speckled pattern significantly more common in sera with SS-DNA precipitins than those without; $p < 0.02$.

(Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number.

There was a significant general association between Ouchterlony precipitins to calf thymus SS-DNA and complement fixing ANA. Complement fixing ANA was present in 21 (44%) of a total 48 sera with Ouchterlony precipitins to calf thymus SS-DNA, but in only 11 (14%) of the 79 sera without such precipitins; $p < 0.001$.

Within the lung disease groups the association was confirmed only in pleuropulmonary SLE where 10 (63%) of the total 16 sera with precipitins had complement fixing ANA, in contrast with only 1 (8%) of the 12 sera without precipitins; $p < 0.01$.

In cryptogenic fibrosing alveolitis 9 (56%) of the total 16 sera with precipitins had complement fixing ANA, in contrast with 9 (29%) of the 31 sera without precipitins, but this was not confirmed significant.

In the remaining lung disease groups the association was not apparent. Although there were a total 16 sera in the asbestosis, silicosis and intrinsic asthma groups, with Ouchterlony precipitins to calf thymus SS-DNA, complement fixing ANA was seen in only 3 sera, all from the silicosis group. Two of these 3 sera also had precipitins.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND REACTIVITY TO CALF THYMUS DEOXYRIBONUCLEOPROTEIN IN ANA POSITIVE SERA

The results are shown in Table 40 with statistical comparisons.

TABLE 39

The extent of association between ANAs precipitating with calf thymus SS-DNA and those fixing complement (in immunofluorescent ANA positive sera).

Disease	SS-DNA precipitins	Complement fixing ANA (No. of sera) Present	Absent	Totals	*Significant association $p \leq$
Pleuropulmonary SLE	Present	10	6	16	
	Absent	1	11	12	0.01
	Totals	11	17	28	
CFA	Present	9	7	16	
	Absent	9	22	31	NS
	Totals	18	29	47	
Asbestosis	Present	0	6	6	
	Absent	0	15	15	
	Totals	0	21	21	
Silicosis	Present	2	7	9	
	Absent	1	12	13	
	Totals	3	19	22	
Intrinsic asthma	Present	0	1	1	
	Absent	0	8	8	
	Totals	0	9	9	
Total patients	Present	21	27	48	
	Absent	11	68	79	0.001
	Totals	32	95	127	

*Statistical analysis using a Fourfold table test for groups < 50 and a Chi square test for groups ≥ 50.

There was a general relationship between Ouchterlony precipitation with calf thymus SS-DNA and reactivity to calf thymus deoxyribonucleoprotein. Antibodies to calf thymus deoxyribonucleoprotein were usually found in sera which also contained Ouchterlony precipitins to calf thymus SS-DNA. They were present in 18 (38%) of the total 47 sera with pricipitins, but in only 8 (10%) of the total 83 sera without precipitins; $p < 0.001$. Seven of the 8 sera with antibodies to deoxyribonucleoprotein but without SS-DNA Outhterlony precipitins were from patients with pleuropulmonary SLE, and the remaining one from a patient with cryptogenic fibrosing alveolitis. In the rare instances where antibodies to deoxyribonucleoprotein were present in the remaining lung diseases, all were associated with precipitins to SS-DNA.

Despite this general relationship, the association was not significant in pleuropulmonary SLE, but in cryptogenic fibrosing alveolitis 5 of the 6 sera with deoxyribonucleoprotein antibodies also had precipitins to SS-DNA; $p < 0.05$.

It is emphasized that the majority of the sera with SS-DNA Ouchterlony precipitins were not associated with antibodies to deoxyribonucleoprotein.

CORRELATION BETWEEN OUCHTERLONY PRECIPITATION TO CALF THYMUS SS-DNA AND OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA IN ANA POSITIVE SERA

The results are shown in Table 41 with statistical comparisons.

There was a highly significant general association between Ouchterlony precipitins to calf thymus SS-DNA and Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA. Of the total 51 sera

TABLE 40

The extent of association between ANAs precipitating with calf thymus SS-DNA and those reacting with calf thymus deoxyribonucleoprotein, (in immunofluorescent ANA positive sera).

Disease	SS-DNA precipitins	Nucleoprotein antibodies (No. of sera) Present	Absent	Totals	*Significant association P ⩽
Pleuropulmonary SLE	Present	10	6	16	NS
	Absent	7	5	12	
	Totals	17	11	28	
CFA	Present	5	10	15	0.05
	Absent	1	28	29	
	Totals	6	38	44	
Asbestosis	Present	1	5	6	
	Absent	0	21	21	
	Totals	1	26	27	
Silicosis	Present	1	8	9	
	Absent	0	13	13	
	Totals	1	21	22	
Intrinsic asthma	Present	1	0	1	
	Absent	0	8	8	
	Totals	1	8	9	
Total patients	Present	18	29	47	0.001
	Absent	8	75	83	
	Totals	26	104	130	

*Statistical analysis using a Fourfold table test for groups ⩽50 and a Chi square test for groups ⩾ 50.

with Ouchterlony precipitins to calf thymus SS-DNA, 33 (65%) also had precipitins to Nuclease SI-treated calf thymus 'DS'-DNA. In contrast only 14 (16%) of the total 87 sera without precipitins to SS-DNA had precipitins to the 'DS'-DNA; $p < 0.001$.

In the separate lung disease groups the closest correlation was in cryptogenic fibrosing alveolitis where 14 (74%) of the 19 sera with SS-DNA precipitins also had 'DS'-DNA precipitins, in comparison with only 7 (21%) of the 33 sera without SS-DNA precipitins; $p < 0.001$. In pleuropulmonary SLE 9 (56%) of the total 16 sera with SS-DNA precipitins also had 'DS'-DNA precipitins, in contrast with only 1 (8%) of the 12 sera without SS-DNA precipitins; $p < 0.05$. In silicosis 8 (89%) of the 9 sera with SS-DNA precipitins also had 'DS'-DNA precipitins, in contrast with 5 (38%) of the 13 sera without SS-DNA precipitins; $p < 0.05$. In asbestosis and intrinsic asthma there was no significant association between the results with SS and 'DS'-DNA.

TABLE 41

The extent of association between ANAs precipitating with calf thymus SS-DNA and those precipitating with Nuclease S1-treated calf thymus 'DS'-DNA (in immunofluorescent ANA positive sera).

Disease	SS-DNA precipitins	'DS'-DNA precipitins (No. of sera) Present	Absent	Totals	*Significant association $P \leq$
Pleuropulmonary SLE	Present	9	7	16	0.05
	Absent	1	11	12	
	Totals	10	18	28	
CFA	Present	14	5	19	0.001
	Absent	7	26	33	
	Totals	21	31	52	
Asbestosis	Present	2	4	6	
	Absent	1	20	21	
	Totals	3	24	27	
Silicosis	Present	8	1	9	0.05
	Absent	5	8	13	
	Totals	13	9	22	
Intrinsic asthma	Present	0	1	1	
	Absent	0	8	8	
	Totals	0	9	9	
Total patients	Present	33	18	51	0.001
	Absent	14	73	87	
	Totals	47	91	138	

*Statistical analysis using a Fourfold table test for groups < 50, and a Chi square test for groups ≥ 50.

IX. ANTIBODIES BINDING WITH 'DOUBLE STRANDED' I^{125}-LABELLED HELA CELL DEOXYRIBONUCLEIC ACID (DS' HELA DNA)

I am most grateful to Dr. Ian Griffiths of the Kennedy Institute of Rheumatology who performed these Farr assay tests.

NORMAL CONTROL STUDIES

The ten ANA positive sera in the group of normal healthy controls were negative in the Farr assay with 'DS' HeLa DNA.

LUNG DISEASE STUDIES

The ANA positive sera used were from twenty-five patients with pleuropulmonary SLE, fifty-one patients with cryptogenic fibrosing alveolitis, twenty-three patients with silicosis, thirty patients with asbestosis and nine patients with intrinsic asthma.

These were the same sera which had been used to investigate ANA titres, immunoglobulin classes of ANA, patterns of ANA immunofluorescence, complement fixing ability of ANA, deoxyribonucleoprotein antibodies, 'DS'-DNA precipitins and SS-DNA precipitins in these lung disease groups. The differences in numbers related to insufficient serum in some instances.

The proportion of the total ANA positive sera with significantly

increased globulin binding to 'DS' HeLa DNA in the different lung disease groups is shown in Table 42, with statistical comparisons in Statistical Table 24, Appendix.

An increase in globulin binding capacity to 'DS' HeLa DNA was extremely rare in lung disease seen in only 11 (8%) of the total 139 ANA positive sera.

In the separate lung disease groups, the frequency of positive binding tests in pleuropulmonary SLE, although not very high, 6 (23%) of the total 26 sera, was higher than in all other disease groups. The only other patients with positive binding were in silicosis, 2 (9%) of the total 23 sera, and in cryptogenic fibrosing alveolitis, 3 (6%) of the total 51 sera.

In view of the small numbers, the increase in pleuropulmonary SLE was significant only in comparison with cryptogenic fibrosing alveolitis and asbestosis.

The Influence of Sex

The results are shown in Table 43 with statistical comparisons. There was a suggestion of a relationship between positive Farr binding to 'DS' HeLa DNA and sex, with a higher frequency of positive binding in females than in males. Overall, positive binding was seen in 8 (15%) of the total 53 females, compared with only 3 (3%) of the total 86 males; $p < 0.02$.

TABLE 42

The proportion of immunofluorescent ANA positive sera with increased Farr-globulin binding to $I^{1.25}$-HeLa cell 'DS' - DNA. (The Farr-binding assay was very kindly performed by Dr. Ian Griffiths, Kennedy Institute of Rheumatology).

Disease	Total number of ANA positive sera	Number with increased Farr-binding to HeLa-cell 'DS' - DNA	
		No.	%
Pleuropulmonary SLE	26	6	23
CFA	51	3	6
Asbestosis	30	0	0
Silicosis	23	2	9
Intrinsic asthma	9	0	0
Total patients	139	11	8

TABLE 43

A comparison of the proportion of immunofluorescent ANA positive sera with increased Farr-globulin binding to I^{125}-HeLa cell 'DS' - DNA in the different sexes. (Statistical comparisons using a test of difference of proportions).

Disease	Sex	Total number of ANA positive sera	Number with increased Farr-binding to HeLa-cell 'DS' - DNA		Significant difference
			No.	%	P ≤
Pleuropulmonary SLE	M	7	1	14	NS
	F	19	5	26	
CFA	M	24	0	0	0.1
	F	27	3	11	
Asbestosis	M	28	0	0	
	F	2	0	0	
Silicosis	M	22	2	9	
	F	1	0	0	
Intrinsic asthma	M	5	0	0	
	F	4	0	0	
Total patients	M	86	3	3	0.02
	F	53	8	15	

However, in the separate lung disease groups this trend was not confirmed in pleuropulmonary SLE, only in cryptogenic fibrosing alveolitis where 3 (11%) of the 27 females had positive binding, but none of the 24 males, $p < 0.1$. However, the frequency of positive binding in females with cryptogenic fibrosing alveolitis could not be shown to differ from that in females with pleuropulmonary SLE.

The Influence of Age

There was no relationship between age and positive binding to 'DS' HeLa DNA.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND ANA TITRES IN ANA POSITIVE SERA

The results are shown in Table 44 with statistical comparisons.

There was a highly significant general association between positive binding to 'DS' HeLa DNA and high titres of ANA immunofluorescence. The mean log. reciprocal ANA titre for the total 11 sera with positive binding was 2.14 (approximately 1/160), compared with a mean of only 1.57 (between 1/20 and 1/40) for the total 128 sera with negative binding; $p < 0.001$.

In pleuropulmonary SLE the 6 sera with positive binding all had

TABLE 44

A comparison of the immunofluorescent ANA titre distributions in sera with and without antibodies binding to 'DS' HeLa cell DNA (Farr assay).

Disease	'DS'-DNA binding antibodies	Total No. of ANA pos. sera	Number and % of total sera at each titre														Mean ANA titre	S.D.	*Sig. diff. P ≤
			Reciprocal of ANA titre																
			10		20		40		80		160		320		640				
			Logarithm of reciprocal of ANA titre																
			1.0		1.3		1.6		1.9		2.2		2.5		2.8				
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%			
SLE	Present	6	0	0	0	0	0	0	0	0	3	50	1	17	2	33	2.45	0.29	0.1
	Absent	20	4	20	0	0	5	25	1	5	4	20	3	15	3	15	1.93	0.63	
CFA	Present	3	0	0	0	0	0	0	1	33	1	33	0	0	1	33	2.30	0.45	0.02
	Absent	48	16	33	5	10	10	21	10	21	4	8	1	2	2	4	1.55	0.50	
ASB	Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
	Absent	30	9	30	10	33	5	17	5	17	0	0	0	0	1	3	1.41	0.41	
SIL	Present	2	2	100	0	0	0	0	0	0	0	0	0	0	0	0	1.00	0.00	
	Absent	21	4	19	9	43	4	19	1	5	2	10	0	0	1	5	1.48	0.45	
AST	Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00	
	Absent	9	2	22	1	11	1	11	4	44	1	11	0	0	0	0	1.63	0.43	
Total patients	Present	11	2	18	0	0	0	0	1	9	4	36	1	9	3	27	2.14	0.64	0.001
	Absent	128	35	27	25	20	24	19	21	16	11	9	4	3	7	5	1.57	0.51	

*Statistical comparisons using an unpaired Student t test.
≠Log. reciprocal.

N.B. % to nearest whole number.

titres of 1/160 or greater in contrast with only 10 (50%) of the 20 sera with negative binding, and the mean log. reciprocal ANA titre in the sera with positive binding was 2.45 (between 1/160 and 1/320), in comparison with a mean of 1.93 (approximately 1/80) for the sera with negative binding; $p < 0.1$. In cryptogenic fibrosing alveolitis the 3 sera with positive binding had ANA titres of 1 in 80 or greater, in contrast with 17 (35%) of the 48 sera with negative binding, and the mean log. reciprocal ANA titre for the sera with positive binding was 2.30 (between 1/160 and 1/320) in comparison with a mean of 1.55 (between 1/20 and 1/40) for the sera with negative binding; $p < 0.02$. However, in silicosis, the two males with positive binding both had very low titres of ANA of only 1 in 10.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND THE PROFILE OF IMMUNOGLOBULIN CLASSES OF ANA IN ANA POSITIVE SERA

The results are shown in Table 45.

Positive binding to 'DS' HeLa DNA was in sera with only two profiles of immunoglobulin classes of ANA. There was a particular association with sera containing a mixture of G, M and A immunoglobulin classes of ANA in 5 (45%) of the total 11 sera with positive binding, in contrast with 16 out of 95 (17%) with this mixture in the group with negative binding; $p < 0.02$. The other 6 (55%) sera had lone IgG ANA, but the prevalence of this profile was not significantly above that in the group with negative binding, 34 out of 95

TABLE 45

A comparison of the profiles of immunoglobulin classes of ANA in sera with and without antibodies binding to 'DS' HeLa cell DNA (Farr assay).

Disease	'DS'-DNA binding antibodies	Total No. of sera	Immunoglobulin class profile (No. and % of total sera)											
			Lone IgG		Lone IgM		IgG/IgM/IgA		IgG/IgM		IgG/IgA		IgM/IgA	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pleuropulmonary SLE	Present	6	2	33	0	0	4	67	0	0	0	0	0	0
	Absent	19	4	21	1	5	2	11	10	58	1	5	0	0
CFA	Present	3	2	67	0	0	1	33	0	0	0	0	0	0
	Absent	28	8	29	7	25	8	29	5	18	0	0	0	0
Asbestosis	Present	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	21	7	33	7	33	2	10	4	19	0	0	1	5
Silicosis	Present	2	2	100	0	0	0	0	0	0	0	0	0	0
	Absent	19	12	63	2	11	3	16	2	11	0	0	0	0
Intrinsic asthma	Present	0	0	0	0	0	0	0	0	0	0	0	0	0
	Absent	8	3	38	0	0	1	12	4	50	0	0	0	0
Total patients	Present	11	6	55	0	0	5	45*	0	0**	0	0	0	0
	Absent	95	34	36	17	18	16	17	25	26	1	1	1	1

*Profile significantly more common in sera with 'DS'-DNA binding antibodies than those without; $p < 0.02$.
**Profile significantly less common in sera with 'DS'-DNA binding antibodies than those without; $p < 0.1$.
(Statistical comparisons using a test of difference of proportions).

N.B. % to nearest whole number.

(36%).

In the separate lung disease groups, the majority of sera with positive binding in pleuropulmonary SLE contained mixed G, M and A ANA's, and the minority lone IgG ANA. In cryptogenic fibrosing alveolitis and silicosis the sera with positive binding had lone IgG ANA, except for one patient with cryptogenic fibrosing alveolitis (in association with scleroderma) who had mixed G, M and A ANA's.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND THE PATTERNS OF ANA IMMUNOFLUORESCENCE IN ANA POSITIVE SERA

The results are shown in Table 46.

For these small numbers of sera with positive binding, no association could be demonstrated between positive binding and any particular pattern of nuclear immunofluorescence. However, it was noted that none of the sera with a nucleolar pattern of nuclear immunofluorescence had positive binding, which was an interesting negative association since both are associated with high titres of ANA immunofluorescence and mixtures of immunoglobulin classes of ANA (although in contrast with positive binding, the nucleolar pattern was associated similarly with mixtures of G, M and A ANA's and with mixtures of G and M ANA's).

TABLE 46

A comparison of the patterns of ANA immunofluorescence in sera with and without antibodies binding to 'DS' HeLa cell DNA (Farr assay).

Patterns	Number of sera with 'DS' HeLa cell DNA binding antibodies							Number of sera without 'DS' HeLa cell DNA binding antibodies						
	Total sera		SLE	CFA	ASB	SIL	AST	Total sera		SLE	CFA	ASB	SIL	AST
	11	(100%)	6	3	0	2	0	94	(100%)	18	28	21	19	8
Homogeneous pattern	8	73	5	1	0	2	0	72	77	15	21	17	13	6
Lone pattern	5	45	3	1	0	2	0	56	60	11	15	15	12	3
In combination	2	18	2	0	0	0	0	16	17	4	6	2	1	3
Peripheral pattern	3	27	2	1	0	0	0	17	18	1	6	5	1	4
Lone pattern	2	18	1	1	0	0	0	9	10	0	4	3	0	2
In combination	1	9	1	0	0	0	0	8	9	1	2	2	1	2
Speckled pattern	2	18	1	1	0	0	0	9	10	5	2	1	0	1
Lone pattern	1	9	0	1	0	0	0	3	3	1	1	1	0	0
In combination	1	9	1	0	0	0	0	6	6	4	1	0	0	1
Nucleolar pattern	0	0	0	0	0	0	0	11	12	5	6	0	0	0
Lone pattern	0	0	0	0	0	0	0	1	1	0	1	0	0	0
In combination	0	0	0	0	0	0	0	10	11	5	5	0	0	0

Statistical comparisons, using a test of difference of proportions, showed no significant differences.

N.B. % to nearest whole number.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND COMPLEMENT FIXING ABILITY IN ANA POSITIVE SERA

The results are shown in Table 47 with statistical comparisons.

There was no significant association between positive binding and the presence of complement fixing ANA. 4 (36%) of the total 11 sera with positive binding (3/6 with SLE and 1/3 with cryptogenic fibrosing alveolitis) also had complement fixing ANA, and 26 (23%) of the 115 with negative binding had complement fixing ANA.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND REACTIVITY TO DEOXYRIBONUCLEOPROTEIN

The results are shown in Table 48 with statistical comparisons.

There was an association between positive binding with 'DS' HeLa DNA and positive reactivity with calf thymus deoxyribonucleoprotein in pleuropulmonary SLE. It was significant that the 6 sera with positive binding also had antibodies to deoxyribonucleoprotein ($p < 0.05$), but 9 (45%) of the remaining 20 sera with negative binding also had antibodies to deoxyribonucleoprotein.

The 3 sera in the cryptogenic fibrosing alveolitis group, and the 2 sera in the silicosis group with positive binding did not have antibodies to deoxyribonucleoprotein. However, one of these silicotic sera was from a patient in whom LE cells had been detected.

TABLE 47

The extent of association between ANAs binding to 'DS' HeLa cell DNA (Farr assay), and those fixing complement (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA binding antibodies	Complement fixing ANA (No. of sera) Present	Absent	Totals	*Significant association P ⩽
Pleuropulmonary SLE	Present	3	3	6	
	Absent	7	13	20	NS
	Totals	10	16	26	
CFA	Present	1	2	3	
	Absent	16	27	43	
	Totals	17	29	46	
Asbestosis	Present	0	0	0	
	Absent	0	22	22	
	Totals	0	22	22	
Silicosis	Present	0	2	2	
	Absent	3	18	21	
	Totals	3	20	23	
Intrinsic asthma	Present	0	0	0	
	Absent	0	9	9	
	Totals	0	9	9	
Total patients	Present	4	7	11	
	Absent	26	89	115	NS
	Totals	30	96	126	

*Statistical analysis using a Fourfold table test for groups ⩽ 50, and a Chi square test for groups ⩾50.

TABLE 48

The extent of association between ANAs binding to 'DS' HeLa cell DNA and those reacting with calf thymus deoxyribonucleoprotein (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA binding antibodies	Nucleoprotein antibodies (No. of sera)			*Significant association
		Present	Absent	Totals	$p \leqslant$
Pleuropulmonary SLE	Present	6	0	6	
	Absent	9	11	20	0.05
	Totals	15	11	26	
CFA	Present	0	3	3	
	Absent	6	34	40	
	Totals	6	37	43	
Asbestosis	Present	0	0	0	
	Absent	2	26	28	
	Totals	2	26	28	
Silicosis	Present	0	2	2	
	Absent	1	20	21	
	Totals	1	22	23	
Intrinsic asthma	Present	0	0	0	
	Absent	1	8	8	
	Totals	1	8	9	
Total patients	Present	6	5	11	
	Absent	19	99	118	0.01
	Totals	25	104	129	

*Statistical analysis using a Fourfold table test for groups < 50, and a Chi square test for groups $\geqslant 50$.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND OUCHTERLONY PRECIPITATION WITH NUCLEASE SI-TREATED CALF THYMUS 'DS'-DNA IN ANA POSITIVE SERA

The results are shown in Table 49, with statistical comparisons.

There was no significant relationship between positive binding to 'DS' HeLa DNA and the presence of Ouchterlony precipitins to Nuclease SI-treated calf thymus 'DS'-DNA in ANA positive sera in any lung disease group. Six (55%) of the total 11 sera with positive binding also had positive precipitins to 'DS'-DNA, and similarly 42 (33%) of the 127 with negative binding had precipitins to 'DS'-DNA.

CORRELATION BETWEEN FARR BINDING WITH 'DS' HELA DNA AND OUCHTERLONY PRECIPITATION WITH CALF THYMUS SS-DNA IN ANA POSITIVE SERA

The results are shown in Table 50, with statistical comparisons.

There was a significant association between positive binding with 'DS' HeLa DNA and the presence of Ouchterlony precipitins to calf thymus SS-DNA. Nine (82%) of the total 11 sera with positive binding to 'DS' HeLa DNA also had precipitins to calf thymus SS-DNA, compared with 41 (33%) of the total 125 sera with negative binding; $p < 0.01$.

In pleuropulmonary SLE it was significant that all 6 patients with

TABLE 49

The extent of association between ANAs binding to 'DS' HeLa cell DNA and those precipitating with Nuclease S1-treated calf thymus 'DS'-DNA (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA binding antibodies	'DS'-DNA precipitins (No. of sera)			*Significant association
		Present	Absent	Totals	$p \leq$
Pleuropulmonary SLE	Present	3	3	6	
	Absent	7	13	20	NS
	Totals	10	16	26	
CFA	Present	2	1	3	
	Absent	19	29	48	
	Totals	21	30	51	
Asbestosis	Present	0	0	0	
	Absent	4	25	29	
	Totals	4	5	29	
Silicosis	Present	1	1	2	
	Absent	12	9	21	
	Totals	13	10	23	
Intrinsic asthma	Present	0	0	0	
	Absent	0	9	9	
	Totals	0	9	9	
Total patients	Present	6	5	11	
	Absent	42	85	127	NS
	Totals	48	90	138	

*Statistical analysis using a Fourfold table test for groups < 50, and a Chi square test for groups ≥ 50.

TABLE 50

The extent of association between ANAs binding to 'DS' HeLa cell DNA and those precipitating with calf thymus SS-DNA (in immunofluorescent ANA positive sera).

Disease	'DS'-DNA binding antibodies	SS-DNA precipitins (No. of sera) Present	Absent	Totals	*Significant association $P \leq$
Pleuropulmonary SLE	Present	6	0	6	0.05
	Absent	9	11	20	
	Totals	15	11	26	
CFA	Present	2	1	3	
	Absent	17	31	48	
	Totals	19	32	51	
Asbestosis	Present	0	0	0	
	Absent	6	21	27	
	Totals	6	21	27	
Silicosis	Present	1	1	2	
	Absent	8	13	21	
	Totals	9	14	23	
Intrinsic asthma	Present	0	0	0	
	Absent	1	8	9	
	Totals	1	8	9	
Total patients	Present	9	2	11	0.01
	Absent	41	84	125	
	Totals	50	86	136	

*Statistical analysis using a Fourfold table test for groups <50, and a Chi square test for groups ≥ 50.

positive binding to 'DS' HeLa DNA also had precipitins to calf thymus SS-DNA ($p < 0.05$) but 9 (45%) of the 20 patients with negative binding also had SS-DNA precipitins. Two of the 3 patients in the cryptogenic fibrosing alveolitis disease group, and one of the 2 in the silicosis group with positive binding also had SS-DNA precipitins.

X. RESULTS OF STUDIES TO DETERMINE WHETHER SPECKLED AND NUCLEOLAR IMMUNOFLUORESCENT STAINING PATTERNS ARE ASSOCIATED WITH ANTIBODIES TO RIBONUCLEATE ANTIGENS

IMMUNOPRECIPITATION TESTS WITH DEPOLYMERIZED TOTAL YEAST RIBONUCLEIC ACID (RNA)

Table 51 shows the results of Ouchterlony double diffusion and counter current electroimmunoprecipitation tests using depolymerized total yeast RNA as antigen, on ANA positive sera from 11 patients with pleuropulmonary SLE or cryptogenic fibrosing alveolitis having speckled and/or nucleolar patterns of nuclear immunofluorescence (sometimes in combination with other patterns), 10 having homogeneous patterns, and on ANA negative sera from 8 normal controls.

The results were negative except for 2 sera with hazy Ouchterlony precipitin lines appearing after 3 days incubation. One had a speckled pattern and the other a homogeneous pattern, and both were from patients with pleuropulmonary SLE.

In summary, antibodies precipitating with depolymerized RNA appear unimportant in pleuropulmonary SLE and cryptogenic fibrosing alveolitis and do not relate to the speckled and nucleolar immunofluorescent ANA staining patterns in these diseases.

TABLE 51

Ouchterlony immunoprecipitation and counter-current electroimmunoprecipitation (EIP) results using depolymerized total yeast ribonucleic acid

Patient	Disease	Pattern of ANA immuno-fluorescence	Ouchterlony immunoprecipitation: Acetate buffer RNA 5 mg/ml	Acetate buffer RNA 1 mg/ml	Phosphate buffer RNA 5 mg/ml	Citrate buffer RNA 5 mg/ml	Counter-current EIP: Barbitone buffer RNA 20 µg/ml
CO	SLE	Sp	+ (Day 3)	0	+ (Day 3)	+ (Day 3)	0
RO	SLE	Sp & H	0	0	0	0	0
OS	CFA	Sp	0	0	0	0	0
RO	CFA	Sp	0	0	0	0	0
BC	CFA	N	0	0	0	0	0
DE	CFA	N	0	0	0	0	0
TH	CFA	N & H	0	0	0	0	0
SM	CFA	N & H	0	0	0	0	0
WH	SLE	N, H & P	0	0	0	0	0
HU	SLE	Sp & N	0	0	0	0	0
MU	SLE	Sp & N	0	0	0	0	0
Controls:							
8 patients	SLE	H	1/8 pos(Day 3)	0/8	1/8 pos(Day 3)	1/8 pos(Day 3)	0/8
2 patients	CFA	H	0/2	0/2	0/2	0/2	0/2
8 subjects	Normal	Neg ANA	0/8	0/8	0/8	0/8	0/8

Sp - Speckled H - Homogeneous N - Nucleolar P - Peripheral Neg - Negative Pos - Positive

CHANGES IN PATTERNS OF ANA IMMUNOFLUORESCENCE ON RIBONUCLEASE AND DEOXYRIBONUCLEASE PRE-TREATED RAT LIVER SECTIONS

Table 52 shows the changes in the patterns of ANA immunofluorescence when indirect immunofluorescent tests for ANA were repeated on rat liver sections pre-treated with ribonuclease to remove ribonuclease sensitive antigens, pre-treated with deoxyribonuclease to remove deoxyribonuclease sensitive antigens, and controls pre-treated with the saline-$MgCl_2$ enzyme diluent.

The observations on speckled patterns in 6 sera, nucleolar patterns in 9 sera, and homogeneous patterns in 20 sera, although few in number, suggested a possible clinical distinction between the speckled and nucleolar patterns in cryptogenic fibrosing alveolitis and those in pleuropulmonary SLE.

In cryptogenic fibrosing alveolitis speckled and nucleolar patterns usually related to ribonuclease sensitive antigens. They were removed by rat liver section pre-treatment with ribonuclease in 4 out of 5 sera, but one nucleolar pattern remained despite all the treatments.

In contrast, in pleuropulmonary SLE these patterns generally related to deoxyribonuclease sensitive or to saline soluble antigens. They were removed by rat liver section pre-treatment with deoxy-

TABLE 52

A comparison of patterns of ANA immunofluorescence on untreated rat liver sections with those on sections pre-treated with ribonuclease, deoxyribonuclease or saline/$MgCl_2$ diluent.

Pattern	Disease	Total sera showing the pattern on untreated sections	No. losing the pattern on sections pre-treated with:				No. retaining the pattern throughout
			Saline/$MgCl_2$ diluent	DNAase only	RNAase only	RNAase or DNAase	
Nucleolar	SLE	5	2	3	0	0	0
	CFA	4	0	0	3	0	1
Speckled	SLE	5	2	2	0	1	0
	CFA	1	0	0	1	0	0
Homogeneous	SLE	14	0	11	0	1	2
	CFA	6	0	5	0	1	0

ribonuclease in 5 instances (5 out of 10), saline pre-treatment in 4 others (4 out of 10), and were not removed by ribonuclease pre-treatment except in one instance (1 out of 10) where a speckled pattern was removed by both ribonuclease and deoxyribonuclease pre-treatment.

The homogenous patterns in pleuropulmonary SLE and cryptogenic fibrosing alveolitis were mainly related to deoxyribonuclease sensitive, not to ribonuclease sensitive antigens (16 out of 20), two were removed by deoxyribonuclease and ribonuclease pre-treatment, and the remaining two were unaffected by all the treatments.

In summary, antibodies reacting with ribonuclease sensitive antigens may frequently relate to the speckled and nucleolar staining patterns in cryptogenic fibrosing alveolitis, but rarely in pleuropulmonary SLE where antibodies to deoxyribonuclease sensitive or saline soluble antigens appear more important.

SUMMARY OF ANTINUCLEAR ANTIBODY RESULTS

The antinuclear antibody findings from sections I to X above are summarized in Table 53.

TABLE 53

Tabulated summary of antinuclear antibody findings: Characteristic features of antinuclear antibodies in human lung diseases.

	Age related	If patterns	If titres (Mean)	Ig classes	Complement fixing (%)	SS-DNA precipitins (%)	DS-DNA binding (%)	Deoxy-NP Ab (%)	LE cells	SS-RNA precipitins
Pleuropul-monary SLE	No	H (Sp)(N)	1/80-1/160	Mainly mixed G M (A)*	39	57	23	61	+	-
CFA	No	H (P)(N)	1/20-1/40	Lone & mixed G M (A)*	34	36	6	12	-	-
Asbestosis	Yes	H (P)	1/20	Mainly lone M (G)	0	22	0	7	-	
Silicosis	No	H (P)	1/20-1/40	Mainly lone G (M)	14	41	9	5	-	
Intrinsic asthma	No	H (P)	1/40	Lone & mixed G M	0	11	0	11	-	

If - Immunofluorescent
Ig - Immunoglobulin
Deoxy-NP - Deoxyribonucleoprotein
*A - In mixtures only
() - Occasionally present, but in the minority

H - Homogeneous
Sp - Speckled
N - Nucleolar
P - Peripheral

XI. RESULTS OF LYMPHOCYTE STUDIES

In these peripheral leucocyte migration tests (LMT) (p.108), not every patient could be studied in full dose response curves to every antigen owing to the limitation imposed by the number of leucocytes obtained from any one reasonably sized blood sample. For this reason the numbers of patients and controls quoted below in Table 55 and in Figures 15-19 do not always correspond exactly to the total number of patient and control studies stated in Table 3 (p 64).

INHIBITION OF LEUCOCYTE MIGRATION WITH NUCLEI, DEOXYRIBONUCLEOPROTEIN AND DNA

Normal Controls

The mean percentage inhibition of leucocyte migration, standard error of the mean (SEM) and the upper limit of the normal range (mean inhibition + 2SD) for two concentrations of each antigen are shown in Table 54. Enhancement values have been excluded from this data and analysed separately because the mechanism of migration enhancement is probably different. The complete results including enhancement are however shown in Figures 15-18. The individual results are shown in Figures 15-17 and in Table 55. Inhibition slightly in excess of normal (mean inhibition + 2SD) was seen in only 1/13 (7.7%) to crude rat liver nuclei, 1/11 (9.1%) to calf thymus deoxyribonucleoprotein and 2/15 (13.3%) to commercial calf thymus 'DS'-DNA.

TABLE 54

Mean percentage inhibition of leucocyte migration (excluding enhancement) and standard error of the mean (SEM) for patient and control groups to nuclei, deoxyribonucleoprotein and DNA

	Nuclei (μg/ml)				Deoxyribonucleoprotein (μg/ml)				DNA (μg/ml)			
	100		50		12.5		6.25		100		50	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SLE	28.0**	4.7	25.8*	8.8	21.7	7.5	19.0$^{\neq}$	5.6	19.0$^{\neq}$	8.3	8.5	4.8
(Corticosteroid treated	11.0	5.5	11.8	6.8	21.8$^{\neq}$	4.3	21.8$^{\neq}$	7.0	8.5	5.2	5.8	3.3)
CFA[+]	11.6	3.4	13.0	5.1	9.6	1.9	9.3	2.8	11.1	2.5	11.6$^{\neq}$	4.0
(Corticosteroid treated	8.0	2.8	3.7	3.0	11.9	3.4	10.7	3.5	21.9$^{\neq}$	7.1	12.1$^{\neq}$	4.5)
Asbestosis	13.1	4.5	13.6	4.4	10.7	4.9	7.8	3.1	6.4	2.9	11.2$^{\neq}$	4.0
Intrinsic asthma	13.0	3.0	6.9	2.9	8.9	2.3	6.9	2.0	6.9	1.3	3.4	1.0
Normal controls	9.0	2.5	7.2	2.3	9.8	3.3	8.6	2.5	7.5	1.4	3.5	1.2
Upper limit of normal range (control mean + 2SD)	27.2		23.4		30.6		25.2		18.4		12.8	

[+]This includes systemic sclerosis with CFA

Mean inhibition significantly greater than in controls: $^{**}p < 0.001$; $^{*}p < 0.01$; $^{\neq}p < 0.025$; $^{\neq}p < 0.05$
(Statistical comparisons using an unpaired Student t test)

Pleuropulmonary SLE

The mean percentage inhibition of leucocyte migration and SEM for the group of untreated patients for each antigen are shown in Table 54. Significant inhibition compared to the control group was demonstrated to all three antigens, the greatest difference being found with the crude nuclei.

The individual results are shown in Figures 15-17 and in Table 55. Values of inhibition greater than the mean + 2SD for controls were seen in 3/5 (60%) using crude nuclei, 1/3 (33.3%) using deoxyribonucleoprotein and 2/6 (33.3%) using DNA.

Plate 21 demonstrates the strongly positive inhibition seen in one of these patients with pleuropulmonary SLE using commercial calf thymus 'DS'-DNA (100 µg/ml) as the antigen, and Plate 22 shows the normal migration in the antigen-free control for comparison.

Cryptogenic Fibrosing Alveolitis

Studied as a group the untreated patients showed significant inhibition of leucocyte migration only to DNA (Table 54).

The individual results were similar (Figures 15-17, Table 55). While only 1/9 (11.1%) showed positive inhibition to crude nuclei 5/11 (45.5%) were positive to DNA. None were positive to deoxyribonucleoprotein.

Inhibition of the *in vitro* migration of peripheral leucocytes from a patient (BS.) with SLE in culture medium containing calf thymus 'DS'-DNA antigen (100 µg/ml) by comparison with the area of migration in antigen-free control medium shown in Plate 22. (Magnification x 10)

Area of *in vitro* migration of peripheral leucocytes from a patient (BS.) with SLE in antigen-free control culture medium. (Magnification x 10)

Plate 21

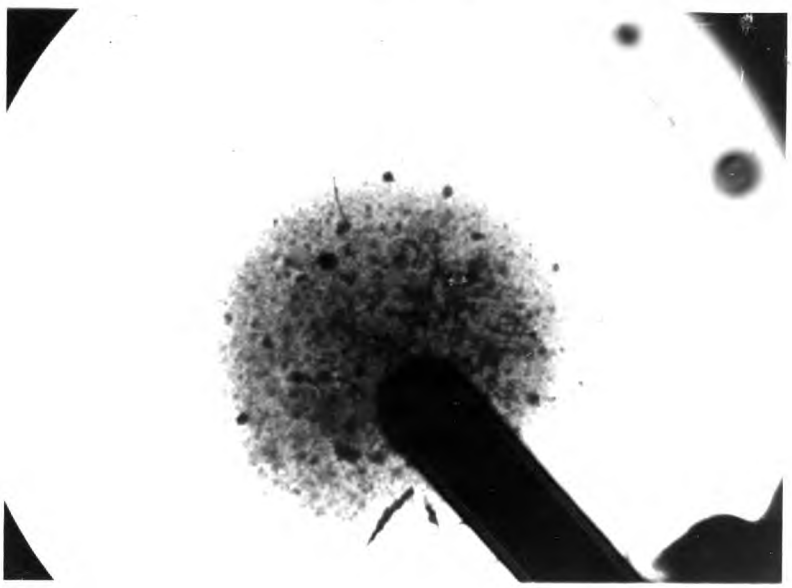


Plate 22



Figure 15

LEUCOCYTE MIGRATION IN SLE, FIBROSING ALVEOLITIS, ASBESTOSIS & NORMAL CONTROL SUBJECTS USING NUCLEI AS ANTIGEN

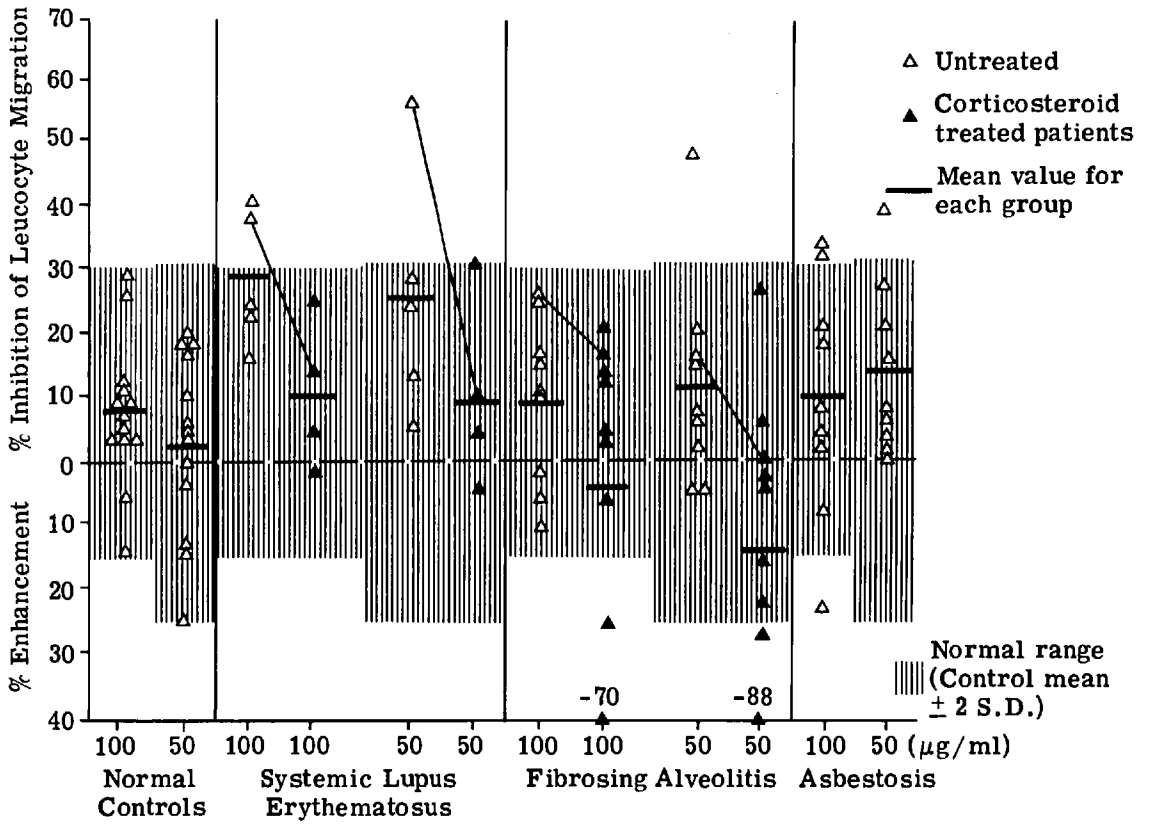


Figure 16

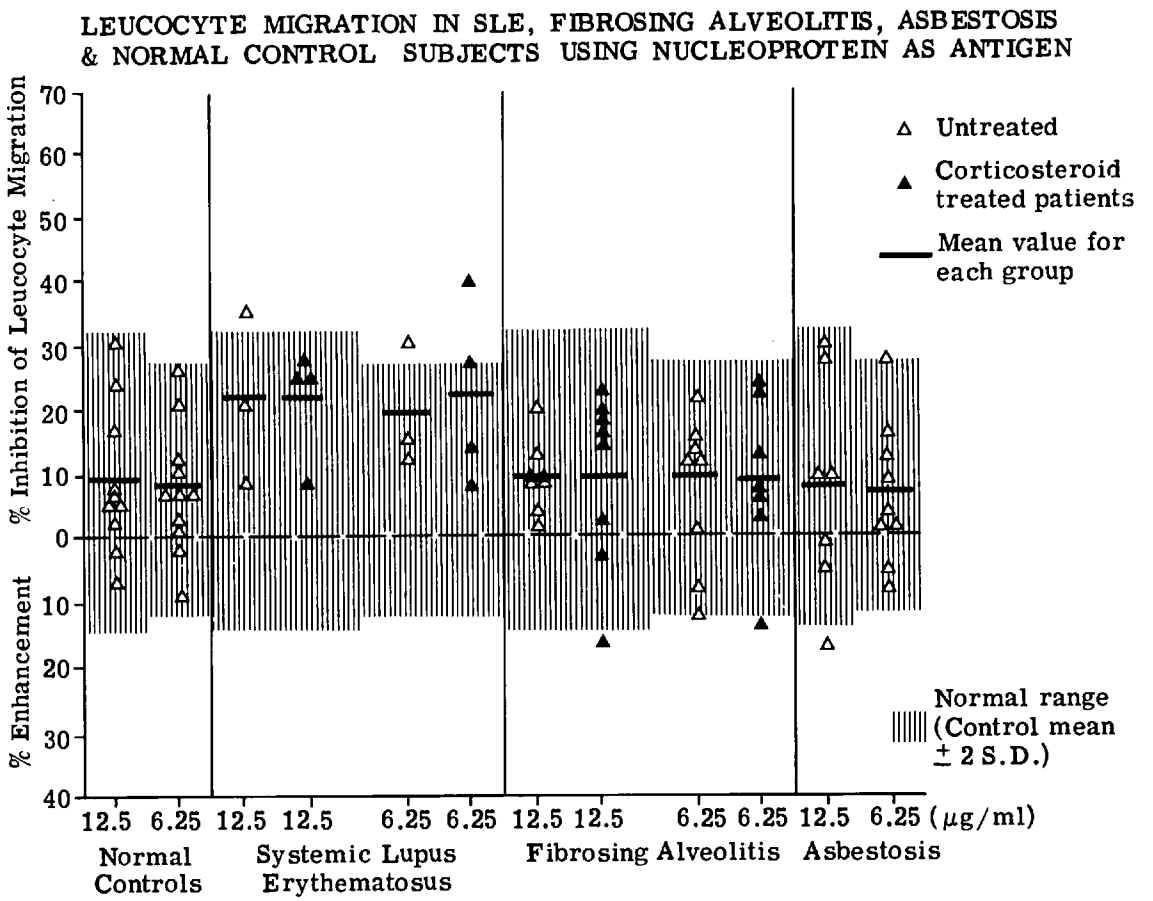

LEUCOCYTE MIGRATION IN SLE, FIBROSING ALVEOLITIS, ASBESTOSIS & NORMAL CONTROL SUBJECTS USING NUCLEOPROTEIN AS ANTIGEN
% Inhibition of Leucocyte Migration
% Enhancement
70
60
50
40
30
20
10
0
10
20
30
40
Untreated
Corticosteroid treated patients
Mean value for each group
Normal range (Control mean ± 2 S.D.)
12.5 6.25 12.5 12.5 6.25 6.25 12.5 12.5 6.25 6.25 12.5 6.25 (μg/ml)
Normal Controls
Systemic Lupus Erythematosus
Fibrosing Alveolitis
Asbestosis

Figure 17

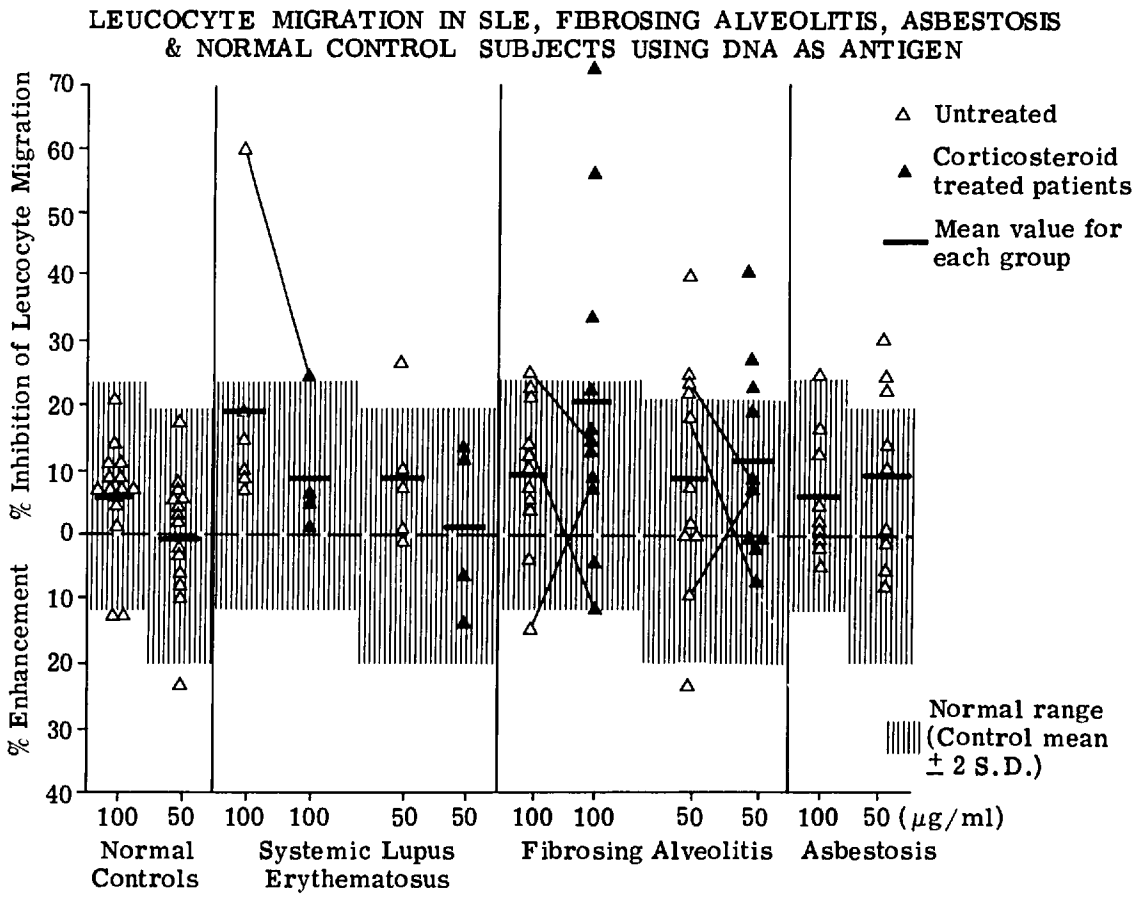

LEUCOCYTE MIGRATION IN SLE, FIBROSING ALVEOLITIS, ASBESTOSIS & NORMAL CONTROL SUBJECTS USING DNA AS ANTIGEN
% Enhancement % Inhibition of Leucocyte Migration
70
60
50
40
30
20
10
0
10
20
30
40
Untreated
Corticosteroid treated patients
Mean value for each group
Normal range (Control mean ± 2 S.D.)
100 50 100 100 50 50 100 100 50 50 100 50 (μg/ml)
Normal Controls
Systemic Lupus Erythematosus
Fibrosing Alveolitis
Asbestosis

TABLE 55

The number of individuals showing inhibition of leucocyte migration greater than the mean + 2SD for the control group (excluding enhancement).

	Crude nuclei positive				Deoxyribonucleoprotein positive				DNA positive			
	Total No.	%	100 µg/ml	50 µg/ml	Total No.	%	12.5 µg/ml	6.25 µg/ml	Total No.	%	100 µg/ml	50 µg/ml
SLE	3/5	60	2/5	3/5	1/3	33.3	1/3	1/3	2/6	33.3	2/6	1/5
(Treated[≠]	1/4	25	0/4	1/4	2/4	50	0/4	2/4	1/4	25	1/4	0/4)
CFA	1/9	11.1	0/9	1/9	0/8		0/8	0/8	5/11	45.5	3/11	5/11
(Treated[≠]	1/9	11.1	0/9	1/9	0/8		0/8	0/7	5/11	45.5	4/11	4/10)
Asbestosis	3/9	33.3	2/9	2/9	1/9	11.1	0/7	1/9	4/9	44.4	1/9	4/9
Intrinsic asthma	0/7		0/7	0/7	0/7		0/7	0/7	0/7		0/7	0/7
Sjögren's syndrome & systemic sclerosis	0/4		0/4	0/4	0/4		0/4	0/4	0/4		0/4	0/4
Controls	1/13	7.7	1/13	0/13	1/11	9.1	0/10	1/11	2/15	13.3	1/15	1/14
Total positive	10/60		5/60	8/60	5/54		1/51	5/53	19/67		12/67	15/65
(% positive	16.7		8.3	13.3	9.3		2.0	9.4	28.4		17.9	23.1)

≠ - Corticosteroid therapy

Asbestosis

The group results were strikingly similar to those for cryptogenic fibrosing alveolitis (Table 54) showing significant inhibition only with DNA.

However the individual results showed in addition to DNA reactivity in 4/9 (44.4%), positive inhibition to crude nuclei in 3/9 (33.3%) and to deoxyribonucleoprotein in 1/9 (11.1%) of cases. (Table 55, Figures 15-17).

Intrinsic Asthma

No significant inhibition of leucocyte migration was seen to any of the three antigens, and the individual values in all cases fell within the normal range.

Systemic Sclerosis and Sjogren's Syndrome

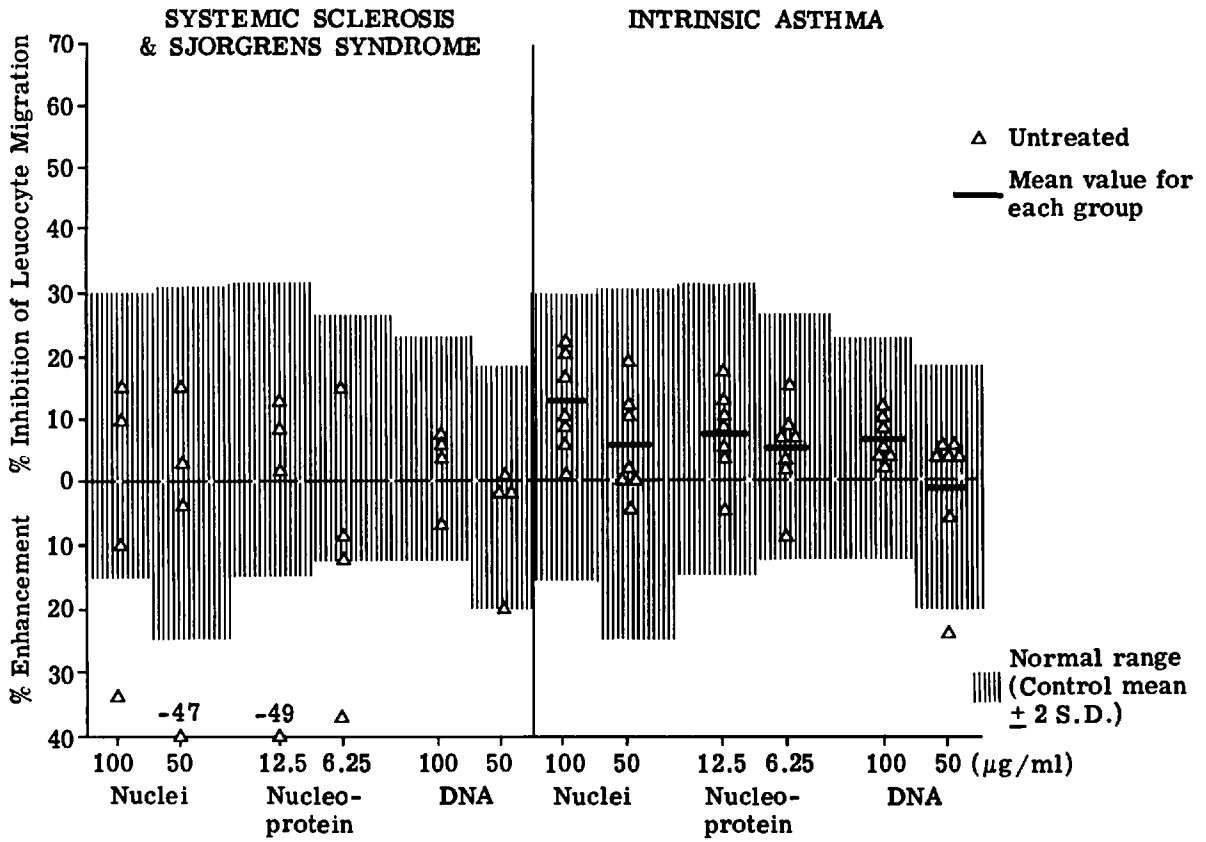
Neither the three patients with systemic sclerosis and pulmonary involvement, nor the one patient with Sjogren's syndrome had evidence of lymphocyte sensitization to any of the three nuclear antigens (Figure 18, Table 55).

ANTIGEN CONCENTRATION

The comparison of the results with two different concentrations of each antigen is shown in Tables 54 and 55. It was found that in many cases, but not exclusively, inhibition was detected better at the lower than the higher antigen dose.

Figure 18

Leucocyte migration in systemic sclerosis and Sjogren's syndrome, and in intrinsic asthma using nuclei, nucleoprotein and DNA as antigen.



ENHANCEMENT OF LEUCOCYTE MIGRATION WITH NUCLEI, DEOXYRIBONUCLEOPROTEIN AND DNA

No significant enhancement of leucocyte migration was demonstrated in any group either by analysing the complete data (Figure 15-18) or analysing enhancement values separately. There were however individuals in some groups showing enhancement values greater than the mean enhancement + 2SD for the controls. In the normal control group 2/13 (15.4%) showed enhancement to crude nuclei, 2/11 (18.2%) to deoxyribonucleoprotein and 3/15 (20%) to DNA. In untreated patients with cryptogenic fibrosing alveolitis 2/11 (18.2%) showed enhancement to DNA. In the Asbestos group 1/9 (11.1%) showed enhancement to crude nuclei and 2/9 (22.2%) to deoxyribonucleoprotein, and one of the seven untreated patients with intrinsic asthma (14.3%) to both deoxyribonucleoprotein and DNA. The one patient with Sjogren's syndrome showed striking enhancement with all three antigens, and of the three patients with systemic sclerosis one showed enhancement to both crude nuclei and deoxyribonucleoprotein and another to deoxyribonucleoprotein only. There was no enhancement at all in the pleuropulmonary SLE group.

Enhancement was seen with high as well as low doses of antigen, and there was no individual showing positive inhibition at the higher dose but enhancement at the lower.

CORRELATION BETWEEN LMT USING CRUDE NUCLEI, AND POSITIVE INHIBITION USING DEOXYRIBONUCLEOPROTEIN AND DNA

The results are shown in Table 56. Leucocytes from 46 subjects were tested with both concentrations of each of the three antigens. Of the 7 with positive inhibition to crude nuclei, 6 (85.7%) gave positive inhibition with DNA and 2 of these (28.6%) also were positive with deoxyribonucleoprotein. Conversely of the 39 subjects without significant inhibition to crude nuclei only 7 (17.9%) were positive to other antigens, 6 (15.4%) to DNA and 2 (5.1%) to deoxyribonucleoprotein one positive to both antigens.

From the results with the purified antigens there was a suggestion of a clinical correlation between positive inhibition to deoxyribonucleoprotein and pleuropulmonary SLE. Of the 4 subjects positive to deoxyribonucleoprotein 3 were pleuropulmonary SLE and 1 was a control subject. A clinical correlation also existed between positive inhibition to DNA alone and cryptogenic fibrosing alveolitis or asbestosis. Of 9 subjects positive to DNA but not deoxyribonucleoprotein 5 were cryptogenic fibrosing alveolitis and 3 were asbestosis. The remaining one was a control.

SINGLE-STRANDED DNA AND DEPOLYMERIZED RNA

Fourteen subjects (2 cryptogenic fibrosing alveolitis, 2 asbestosis, 4 intrinsic asthma, 3 systemic sclerosis, 1 Sjogren's syndrome and 2

TABLE 56

The correlation between LMT results (excluding enhancement) using crude nuclei as antigens and positive inhibition using deoxyribonucleoprotein and DNA.*

	Leucocyte Migration											
	Crude nuclei positive	Diseases				Crude nuclei negative	Diseases					
		SLE	CFA	ASB	C		SLE	CFA	ASB	AST	SJ	C
	7	2	2	2	1	39	5	13	5	7	1	8
DNA only	4	0	2	2	0	5	0	3	1	0	0	1
DNA and Nucleoprotein	2	2	0	0	0	1	0	0	0	0	0	1
Nucleoprotein only	0	0	0	0	0	1	1	0	0	0	0	0
Neither	1	0	0	0	1	32	4	10	4	7	1	6
Total positive to DNA and/or nucleoprotein	6	2	2	2	0	7	1	3	1	0	0	2

SLE - Systemic lupus erythematosus
CFA - Cryptogenic fibrosing alveolitis
ASB - Asbestosis
AST - Intrinsic asthma
SJ - Sjögren's syndrome
C - Control

*This table includes data on 46 subjects where tests to all the three nuclear antigens were complete.

controls) were studied with calf thymus single stranded (denatured) DNA and depolymerized total yeast RNA as well as the usual 3 antigens.

There was no significant inhibition to RNA in any case tested. Positive inhibition to calf thymus SS-DNA was seen in only one subject, a cryptogenic fibrosing alveolitis patient who also showed positive inhibition with calf thymus 'DS'-DNA. The remaining subjects were negative with all five antigens.

CORRELATION BETWEEN ANA TITRE AND INHIBITION OF LEUCOCYTE MIGRATION

The results are shown in Figure 19. A range of immunofluorescent ANA titres was found in each of the disease groups studied. No relationship was found between the ANA titre and the presence or absence of significant inhibition of leucocyte migration.

CORRELATION BETWEEN LMT AND ANTIBODY RESULTS USING DNA AND DEOXYRIBONUCLEOPROTEIN

The results are shown in Table 57. There was no correlation between presence or absence of serum antibody and presence or absence of significant inhibition of leucocyte migration using either calf thymus 'DS'-DNA or calf thymus deoxyribonucleoprotein in untreated patients.

Figure 19

ANTINUCLEAR ANTIBODY TITRE & INHIBITION OF LEUCOCYTE MIGRATION (EXCLUDING ENHANCEMENT) TO CRUDE NUCLEI NUCLEOPROTEIN OR DNA

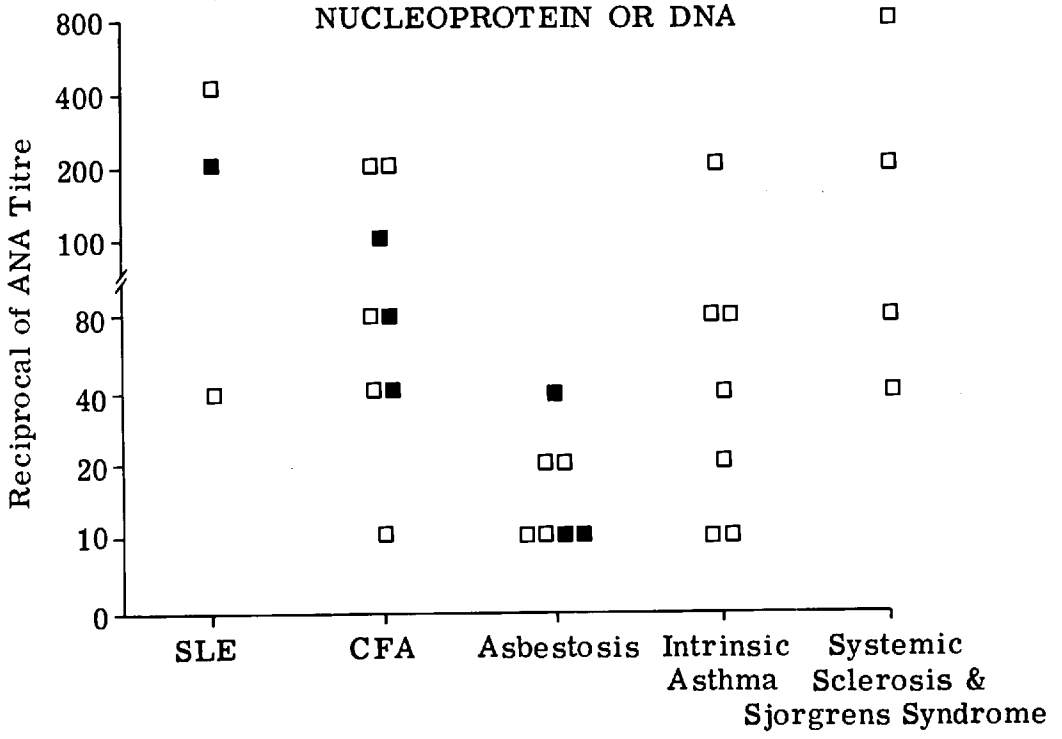


TABLE 57

A correlation of LMT (excluding enhancement) and antibody results using DNA and deoxyribonucleoprotein (untreated patients only)

	Total no. of patients	LMT	Serum antibody: SLE +	SLE −	CFA +	CFA −	ASB +	ASB −	AST +	AST −	SJ +	SJ −	Total +	Total −	Significant association*
DNA	34	+ 11	2	0	2	3	1	3	0	0	0	0	5	6	NS
		− 23	1	3	3	3	3	2	0	7	0	1	7	16	
Nucleoprotein	27	+ 2	0	1	0	0	0	1	0	0	0	0	0	2	
		− 25	1	1	0	8	1	7	1	6	0	0	3	22	

*Statistical analysis using a Fourfold Table Test
NS - No significant association

Positive inhibition was observed in cases with and without serum antibody, and there were many patients with serum antibody but without significant inhibition of leucocyte migration.

EFFECT OF CORTICOSTEROID TREATMENT

Groups of patients with pleuropulmonary SLE and cryptogenic fibrosing alveolitis who were receiving corticosteroids at the time of study have been included in Tables 54 and 55 and in Figures 15-17. The mean migration inhibition in the treated groups was frequently lower than in the corresponding untreated group for the same dose of antigen. The return towards normal was most marked with crude nuclei and least with deoxyribonucleoprotein.

In addition to a reduction in inhibition, there were individuals in the treated cryptogenic fibrosing alveolitis group showing significant enhancement of migration to crude nuclei and deoxyribonucleoprotein which had not been seen in the untreated group. Enhancement was shown in 3/9 (33.3%) to crude nuclei, and 1/8 (12.5%) to deoxyribonucleoprotein.

In occasional patients tests were performed before and after treatment (indicated by the connecting lines Figures 15-17). These too showed a consistent reduction in the degree of inhibition following corticosteroid treatment. Interestingly, one patient with cryptogenic fibrosing alveolitis showing enhancement with DNA before treatment returned to normal afterwards.

SUMMARY OF LYMPHOCYTE RESULTS

The lymphocyte findings from section XI above are summarized in Table 58.

TABLE 58

Tabulated summary of lymphocyte findings: Characteristic features of lymphocyte sensitisation to antigens of cell nuclei in human lung diseases.

	Percentage of patients with evidence of leucocyte inhibition to:		
	Whole nuclei (%)	Deoxyribo-nucleoprotein (%)	DNA (%)
Pleuropulmonary SLE	60	33	33
CFA	11	0	46
Asbestosis	33	11	44
Intrinsic asthma	0	0	0

CHAPTER 5

DISCUSSION

GENERAL DISCUSSION

The thesis proposal that nuclear antigens stimulate different types of immune response in lung disease according to the distinctive clinical circumstances originated from data which suggested variability in the prevalence of immunofluorescent ANA in different lung diseases. The early confirmation that this was not the consequence of age or sex variation was an encouraging start to this study and justified continuation with the detailed investigations. These have provided further support for the thesis proposal.

ANA positive patients with pleuropulmonary SLE, cryptogenic fibrosing alveolitis (CFA), asbestosis, silicosis and intrinsic asthma have been shown to differ in the numbers and types of nuclear antigens involved, and the numbers and types of antibodies stimulated. It has also been shown that cellular as well as humoral immune responses to nuclear antigens are stimulated in some patients. However, in many other patients the humoral response was not accompanied by a cellular response. The clinical distinctions were usually characteristic combinations of features, and only rarely was any single characterising feature demonstrated.

The alternative idea that responses to nuclear antigens in lung disease are stimulated in a general way as a consequence of any

kind of extensive tissue destruction in the lung which causes the release of nuclear antigens would seem unlikely in the light of the present observations.

SENSITISATION TO NUCLEAR ANTIGENS IN NORMAL CONTROLS.

Antinuclear Antibodies

The normal control study served to validate the modification of the indirect immunofluorescent screening method used here to detect ANA since the findings were in keeping with those of other workers using their own modifications of the method. The overall very low prevalence of ANA in randomly selected normal controls was confirmed (Alexander Bremner & Duthie, 1960; Ward, Johnson & Holborrow, 1964; Feltkamp, 1966), as was the progressive increase in ANA prevalence with advancing age, with a greater increase in females than in males (Beck, 1963; Cannat and Seligmann, 1965; Whittingham et al, 1969). In this study the maximum prevalence noted was 25% in the 70 plus age group, but others have reported that the increase continues as high as 50% for an 80 plus age group (Cannat and Seligmann, 1965). These observations, and reports of an increased production of other anti-tissue antibodies with advancing age, particularly thyroid microsomal antibodies, and gastric parietal cell antibodies (Serafini, Torrigiani & Masala, 1964; Whittingham et al, 1969; Hooper et al, 1972), suggest age-related breakdown or by-pass of tolerance in

the normal control population.

Several explanations have been proposed for breakdown, or by-pass, of tolerance to some tissue antigens with age. One suggestion is that subjects living to old age have more opportunities for repeated incidental exposure to tissue antigens released, for example, during inflammatory reactions of various kinds, or as a consequence of infection, or direct mechanical trauma. Certainly transient antinuclear antibody has been reported following exposure to bacterial antigens in both man (Bonomo et al, 1965) and in experimental animals (Christian, De Simone & Abruzzo, 1965), and has also been reported in some patients after severe burn injury (Quismorio, Bland & Friou, 1971). These are all procedures which not only may cause the release of tissue antigens but might also induce their structural modification. It has also been suggested that aging may be associated with a central breakdown rather than a by-pass of tolerance, possibly due to the failure of T-cell suppressor function (Allison, Denman & Barnes, 1971). However such a theory cannot satisfactorily explain preferential breakdown of tolerance to some tissue antigens but not others. Another explanation may be that autologous antigens undergo changes with age due to cellular deterioration and malfunction and are thereby recognised as foreign (Ram, 1967). Since the various cell types of the body probably deteriorate at different rates this could explain the preferential production of some tissue antibodies over others. Evidence of a change in the structure of cellular

DNA with age has recently been acquired by Chetsanga and his co-workers (1975) who have discovered that DNA extracted from liver cells of young mice aged up to 15 months exists mainly in normal double-helical form, but by the age of 30 months about a quarter of the total liver cell DNA is in single-stranded form. If this should be confirmed in humans, the persistence of abnormally high quantities of SS-DNA would provide one possible type of autologous antigen appearing with age which might be recognised as foreign and stimulate ANA production. In the present study Ouchterlony precipitins to SS-DNA were identified in only one of the 10 normal controls who had age-related ANA. It is possible that the Ouchterlony immunoprecipitation method is not sufficiently sensitive to demonstrate small quantities of antibodies to SS-DNA. Koffler et al (1969) using a haemagglutination method identified antibodies to SS-DNA in 3% of normal healthy controls.

The age-related ANA's in the ten normal controls were characteristically of very low titre, never exceeding 1 in 40. Similarly low titres have been described for age related normal control ANA's by Cannat and Seligmann (1965). The immunoglobulin class of ANA was always IgM, and usually the only class involved except in one female with both IgM and IgG ANA. In other studies of normal control ANA's only polyvalent antisera have been used. It is of interest that IgM is usually responsible for the major part of ANA activity in various diseases associated with low titre ANA's (Baum and Ziff, 1962; Gonzalez and Rothfield, 1966).

The patterns of immunofluorescence associated with the normal control ANA's were either speckled or peripheral. Other workers have described immunofluorescent ANA patterns in normal controls as either speckled or diffuse (Cammarata, Rodnan & Fennell, 1967; Rothfield and Rodnan, 1968). However, as has been previously emphasised, immunofluorescent patterns are very variable even within the four main descriptive types, and may be interpreted differently by different observers. The observation in this study that females tend to have a speckled pattern while males show a peripheral pattern, may be of little significance for such small numbers. However, it is possible that the two pattern types indicate the participation of at least two nuclear antigens, with the added possibility that one is important in females, but the other in males.

The attempts to identify some of the nuclear antigens reacting with normal control ANA's were unsuccessful. Antigens examined were deoxyribonucleoprotein, the main component of the nuclear chromatin body, double-stranded DNA (DS-DNA), the gene bearing region of deoxyribonucleoprotein, and SS-DNA which normally exists only transiently in the nucleus. Except for the one normal control with positive Ouchterlony precipitins to calf-thymus SS-DNA, none of these macromolecules could be confirmed to stimulate ANA production in this small number of normal controls.

Finally, none of the normal control ANA's could fix complement.

It has been reported that IgM ANA may only fix complement at 37°C, unlike IgG ANA's which may fix complement at both 4°C and 37°C (Cohen et al, 1971). Since the complement fixation stage of the immunofluorescent method used here was carried out at 37°C, the most likely conclusion is that the IgM ANA's of normal controls are non-complement fixing.

Lymphocyte Sensitisation

Regrettably it was not possible to study lymphocytes from the 10 normal controls with age-related ANA, but 16 normal healthy controls who did not have immunofluorescent ANA also showed only minimal inhibition of peripheral leucocyte migration in the presence of nuclear antigens by comparison with migration in antigen-free controls. Nuclear antigens examined were purified rat liver nuclei, calf thymus deoxyribonucleoprotein, and calf thymus 'DS'-DNA. The slight inhibition in some instances may reflect low levels of lymphocyte sensitisation but it is more probable that this is a non-specific antigen-related effect, for example, due to slight changes in pH, or viscosity. It is unlikely to be the result of a cytotoxic effect of antigen since the viability studies with trypan blue indicated that the antigens were not cytotoxic over the dose ranges selected for the tests.

SENSITIZATION TO NUCLEAR ANTIGENS IN 'PLEUROPULMONARY' SLE

Antinuclear Antibodies

The ANA's in this special group of patients with 'pleuropulmonary' SLE not only differed from those in elderly normal controls, but also differed in many respects from the ANA's in all the other lung diseases. However, in general they resembled those in other series of patients with SLE.

Like classical SLE, lupoid hepatitis and scleroderma (Ritchie, 1967) the patients with 'pleuropulmonary' SLE usually had high ANA titres and a greater number of patients had titres equal to or exceeding 1 in 160 than in any other lung disease group.

Deoxyribonucleoprotein antibodies

The most striking distinguishing feature separating patients with 'pleuropulmonary' SLE from those with other lung diseases was the presence of antibodies reacting with deoxyribonucleoprotein in 61% (17 out of 28) of the patients. Positive reactions with deoxyribonucleoprotein were extremely rare in the other lung diseases. Friou (1958; 1967) using an immunofluorescent method similar to that used in the present study reported that antibodies reacting with deoxyribonucleoprotein occur in nearly all patients with SLE but only very occasionally in other diseases.

The frequent positive results of immunofluorescent tests with

deoxyribonucleoprotein are in keeping with the positive LE cell tests in 86% (24 out of 28) of the patients with 'pleuropulmonary' SLE. LE cell factor can be demonstrated in about 80% of cases with SLE (Barnett and Rothfield, 1969) and there is evidence that it is an antibody to deoxyribonucleoprotein (Holman and Deicher, 1959). However, the very occasional patients positive in immunofluorescent tests with deoxyribonucleoprotein in the other lung disease groups were negative in LE cell tests with the exception of one patient with CFA. Nor were there any clinical features suggestive of SLE except in this one patient.

There are a number of reports which suggest a possible role for antibodies to deoxyribonucleoprotein in pathogenesis. Robitaille (1974) has recently reported that in serial studies of patients with SLE the presence of antibodies to nucleoprotein and/or native DNA paralleled renal disease activity as reflected by the degree of proteinuria. In an earlier study Koffler, Schur and Kunkel (1967) identified antibodies reacting with deoxyribonucleoprotein as commonly as those reacting with DNA in eluates from isolated glomeruli of kidneys from patients with SLE nephritis after treatment with deoxyribonuclease. However, using an Ouchterlony immunodiffusion method Schur and Sandson (1968) were unable to demonstrate any association between the presence of precipitating antibodies to soluble deoxyribonucleoprotein in 49% of 96 patients with SLE and disease activity or clinical nephritis.

In our group of patients with 'pleuropulmonary' SLE, although a majority of sera (17 out of 28) contained antibodies to deoxyribonucleoprotein, only seven patients had possible minimal renal involvement (Holgate et al, 1976), six of these had antibodies to deoxyribonucleoprotein. Immune complexes involving deoxyribonucleoprotein have recently been implicated in synovial injury in rheumatoid arthritis (Robitaille, Zvaifler and Tan, 1973). Joint changes were present in eleven of our patients with 'pleuropulmonary' SLE and eight had deoxyribonucleoprotein antibodies.

Finally, the absence of deoxyribonucleoprotein antibodies from sera with lone IgM ANA and their presence only in sera with either IgG ANA or mixtures of IgG ANA with other immunoglobulin classes suggests that IgG may be important in reactivity to deoxyribonucleoprotein in 'pleuropulmonary' SLE. The involvement of IgG in deoxyribonucleoprotein reactivity in SLE has been confirmed by several workers (Friou and Teague, 1963).

Deoxyribonucleic acid antibodies

Patients with 'pleuropulmonary' SLE differed from those in all other lung diseases in one other respect. Farr assays aimed at demonstrating antibodies reacting with double-stranded DNA, were positive in 24% of patients with 'pleuropulmonary' SLE, but very rarely positive in patients with other lung diseases. This finding

is in keeping with results obtained generally using this type of DNA-binding test which has so far shown considerable diagnostic specificity for SLE (Wold et al, 1968; Pincus et al, 1969; Hughes, Cohen and Christian, 1971; Glass et al, 1973). The 24% prevalence of positive binding in our patients with 'pleuropulmonary' SLE was, however, lower than has previously been reported in groups of patients with SLE referred to other specialists. Binding is usually observed in between 68% to 90% of patients the lower figure referring to cases studied during clinical remission and the higher figure referring to patients with progressive SLE or studied during a period of acute clinical relapse (Peltier et al, 1974).

The lower than expected prevalence of positive binding tests in our series of patients with 'pleuropulmonary' SLE has now been twice reconfirmed using two slightly different modifications of the binding assay, one employing I^{125} HeLa cell DNA as in this study but using double-antibody globulin precipitation (Holgate et al, 1976), and the other using H^3 calf thymus DNA (Haslam et al, 1976). Not only is the incidence of positive binding low, but the binding levels are also more in keeping with figures obtained by others for cases of SLE in clinical remission (Peltier et al, 1974). The low incidence of positive binding in this series, together with an additional demonstration of normal or high serum complement

(C_3 and CH_{50}) levels, has been speculatively linked with the low incidence of renal disease and a slowly progressive subacute type of disease in these patients with 'pleuropulmonary' SLE compared with more usual groups of SLE patients (Holgate et al, 1976). In many other SLE series active disease has been found to be associated with a rise in the titres of antibodies to DNA as measured by binding, complement fixation or other methods together with a fall in the serum complement levels (Schur and Sandson, 1968; Webb, Whaley and Lee, 1974). Follow-up studies in the few 'pleuropulmonary' SLE patients with positive binding would be of interest since they may be subject to more frequent and more severe periods of clinical relapse than those with negative binding, and binding levels may fluctuate accordingly.

Positive binding with DNA distinguished 'pleuropulmonary' SLE from the other lung diseases but an explanation must now be found for the positive Ouchterlony precipitins to DNA in all the disease groups except intrinsic asthma. Many different methods are currently in use to demonstrate antibodies to DNA including complement fixation (Robbins et al, 1957) haemagglutination (Jokinen and Julkunen, 1965), counter-current electroimmunoprecipitation (Davis, 1971) and Crithidia luciliae immunofluorescence (Aarden, de Groot and Feltkamp, 1975) as well as the radioimmunoassay and Ouchterlony immunodiffusion methods. Variable results are frequently obtained using different methods for reasons which are not fully understood (Davis, 1975).

There are several possible explanations for the different Farr assay and Ouchterlony precipitation results. One possibility is that the Ouchterlony method may be demonstrating non-immunological precipitation of negative charged DNA with positively charged serum components such as C1q (Agnello et al, 1969). This, however, seems an unlikely explanation in view of the confirmation of the results using heat-inactivated sera and counter-current immunoprecipitation to distinguish immunological from non-immunological precipitation, the demonstration of immunoglobulin in the Ouchterlony precipitin lines using I^{125}-anti human immunoglobulin, and the immunoelectrophoretic analysis which suggested the involvement of IgG immunoglobulin. Perhaps one of the most important reasons for the variable results is that different types of DNA were used as the test antigens. The physicochemical studies suggested that the HeLa cell DNA used in the Farr assays had suffered no, or minimal, damage to the double-stranded helix during preparation, but that some single-stranded breaks were present in the calf thymus DNA purchased for use in the Ouchterlony tests. It is therefore possible that the additional positive results obtained using the Ouchterlony method may relate to antibodies reacting with single-stranded rather than double-stranded DNA. In keeping with this suggestion is the generally close correlation of the Ouchterlony test results with calf thymus 'DS' and SS-DNA in all the diseases. These findings also suggest that Nuclease S1 treatment is not a reliable method for achieving complete removal of SS-DNA contamination from damaged double-stranded DNA. Recently it has been shown that DNA-binding

levels decrease linearly with decreasing Molecular Weight of DNA test antigen (Geisert et al, 1975), and infrequent binding could be due to the use of low Molecular Weight DNA. However, the HeLa cell DNA used in these binding studies was of high Molecular Weight (11.56×10^6). Aarden (personal communication) has recently suggested that precipitation with DNA may be especially due to IgM antibodies but DNA-binding due to IgG antibodies. However, the demonstration of IgG, but never IgM DNA precipitins in the lung disease sera so far examined suggests that this is not the explanation for the different results in this study. Finally the significant association of the positive binding with high ANA titres suggests that a quantitative explanation for the different binding and precipitation results should not be disregarded.

If it should be confirmed that the Farr tests are demonstrating antibodies to DS-DNA in 'pleuropulmonary' SLE, it is possible that they may also cross-react with SS-DNA. We have recently shown that the positive binding in these patients can in all instances be removed by prior absorption of sera with SS-DNA (Haslam et al, 1976). The ability of SS-DNA to totally inhibit reactivity to native DNA in SLE has been previously reported (Stollar and Levine, 1961; Arana and Seligmann, 1967; Koffler et al, 1969). There is also evidence of another type of antibody in many SLE sera and in ANA positive sera from various other diseases which reacts with,

and is inhibited by, only SS-DNA (Koffler et al, 1969; Tan and Natali, 1970). It is possible that the majority of sera in this study with positive precipitins but negative binding have a similar type of antibody. Designation of the DNA precipitins in lung diseases as DS-DNA specific, DS/SS-DNA cross-reactive or SS-DNA specific must, however, await further studies.

In the past most attention has been given to investigating the role of native-DNA antibodies in the pathogenesis of the kidney and skin lesions of patients with SLE, mainly because antibodies reactive with DS-DNA have shown considerable diagnostic specificity for SLE. In addition their levels have fluctuated in close agreement with serum complement levels and have provided a method for monitoring the activity of the renal disease (Schur and Sandson, 1968). Antibodies to SS-DNA were considered probably of lesser importance in SLE since high levels are frequently maintained during clinical remission and in addition they frequently occur in diseases other than SLE. However, these observations in lung disease suggest that if antibodies to DNA play any significant role in pathogenesis, those to SS-DNA may be generally more important than those to DS-DNA even in 'pleuropulmonary' SLE. Recent evidence of immune-complexes involving SS-DNA and antibody to SS-DNA in glomeruli of 8 out of 9 patients with SLE nephritis, apparently able to activate complement via the primary pathway (Koffler, Agnello and Kunkel, 1974) has suggested that antibodies to SS-DNA may also play a role in pathogenesis. Koffler et al (1973) also reported a high incidence

of free SS-DNA occurring in greatest concentration in sera of patients with SLE, and in high incidence but lower levels in the sera of patients with rheumatoid arthritis, and in several other diseases where a low incidence of antibodies to SS-DNA is observed, suggesting that in all these diseases a situation conducive to SS-DNA anti-SS-DNA immune complex formation might arise. Finally, it has been reported that New Zealand mice rendered tolerant to denatured DNA fail to make antibody to denatured DNA and fail to develop chronic membranous glomerulonephritis (Borel, Lewis and Stollar, 1973). Similar findings have also been reported by Parker, Hahn and Osterland (1974).

It is not known why all eleven patients with positive DS-DNA binding whatever their disease, also had antibodies to deoxyribonucleoprotein. Purely speculative proposals are that both types of response may be stimulated by viral antigens (p.), or perhaps the DS-DNA binding antibodies may also cross-react with the DNA of deoxyribonucleoprotein.

Complement fixing ANA

While 'pleuropulmonary' SLE was distinguished from all other lung diseases by the presence of antibodies reacting with deoxyribonucleoprotein, LE cells and antibodies binding with DS-DNA, other features were noted in this study which provide immunological links between the ANA's of 'pleuropulmonary' SLE and those of certain other lung diseases, and at the same time distinguish them

from the remaining lung diseases. One observation falling into this category and linking 'pleuropulmonary' SLE with CFA, was the finding of complement fixing ANA in the serum of 39% of the ANA positive patients with 'pleuropulmonary' SLE and 34% of the patients with CFA. Complement fixing ANA's were absent from all the other lung disease groups except for just 3 (14%) of 22 ANA positive silicotic sera.

Complement fixation is known to be a variable property of the ANA's in SLE (Tojo and Friou, 1968) and it has been said to correlate well with the presence of IgG1 and IgG3 subclasses of ANA (Puritz et al, 1973). In this study complement fixing ANA was particularly associated with mixtures of IgG, IgM and IgA ANA's and was also found in sera with lone IgG ANA but there was a significant absence in sera with lone IgM ANA. It would now seem appropriate to look for a relationship between IgG subclasses and complement fixing ability of the ANA's in 'pleuropulmonary' SLE and CFA.

Complement fixing antibodies to deoxyribonucleoprotein have been described in many SLE sera (Holman and Deicher, 1959). However, in this study deoxyribonucleoprotein antibodies were detected in sera with and without complement fixing ANA and similar findings

have been obtained by Rothfield and Stollar (1967). There have also been many reports of complement fixing antibodies to DNA in SLE (Robbins et al, 1957; Seligmann and Milgrom, 1957; Schur and Sandson, 1968). The evidence of an association between complement fixing ANA and SS-DNA precipitins in 'pleuropulmonary' SLE and CFA suggests a possibility that some SS-DNA precipitins in these diseases may fix complement. If so they may be distinct from the SS-DNA precipitins in asbestosis, silicosis and intrinsic asthma which occur in sera with non-complement fixing ANA.

ANA's with complement fixing ability may be particularly well able to take part in tissue damaging reactions.

Immunoglobulin classes of ANA

The multiple immunoglobulin classes of ANA in 'pleuropulmonary' SLE which included IgG, IgM and IgA resemble the findings in other groups of patients with SLE (Barnett et al, 1964; Gonzales and Rothfield, 1966), and Kantor, Van Herle and Barnett (1970) have also reported IgD ANA in SLE sera. The mixtures of immunoglobulin classes of ANA in individual sera which were also common in CFA and intrinsic asthma may reflect multiple stimulatory antigens. There was evidence of a number of different antigens in 'pleuropulmonary' SLE but not in the other two diseases. Prolonged antigenic stimulation is another possible explanation for a multiple immunoglobulin class response but in lung diseases, even in asbestosis where patients tend to produce a lone immunoglobulin

class of ANA, the ANA's appear to persist (Turner-Warwick, 1974) suggesting persistent antigenic stimulation.

There has been some recent interest in the theory that immediate-type hypersensitivity reactions mediated by reaginic antibodies could be one mechanism for damage to cell membranes with consequent increase in capillary permiability which might allow access of circulating immune complexes to the tissues in patients with SLE (Knicker and Cochrane, 1968). For this reason the tendency for mixtures of IgG and IgM ANA to occur mainly in patients with 'pleuropulmonary' SLE and intrinsic asthma was of interest. We have recently followed up this slight suggestion of an immunological link between 'pleuropulmonary' SLE and intrinsic asthma by looking for IgE ANA (Haslam, Soutar and Turner-Warwick, 1976). However, while we have identified ANA's reacting with an anti-human IgE antiserum this did not establish a link between SLE and intrinsic asthma, nor are we yet convinced that the antiserum was reacting specifically with IgE.

Miyawaki and Ritchie (1974) have described IgE ANA in 48% of patients with SLE and in 58% of patients with procainamide induced SLE, but in only 5% of patients with rheumatoid arthritis. In addition they described IgE ANA in 56% of sera with antinucleolar

ANA from patients with systemic sclerosis or atypical illnesses associated with Raynaud's phenomenon. Attempts to obtain evidence for a role for IgE in various nephropathies using immunofluorescent methods have so far been unsuccessful (Roy, Westberg and Michael, 1973; Bennich et al, 1974).

Immunofluorescent staining patterns

The failure to demonstrate the peripheral pattern of staining in 'pleuropulmonary' SLE despite frequent descriptions of the pattern in other groups of patients with SLE, particularly those who are acutely ill (Gonzalez and Rothfield, 1966; Casals, Friou and Teague, 1963), is another immunological observation in keeping with the conclusion of Holgate et al, (1976) that 'pleuropulmonary' SLE may be a sub-acute form of the disease. The peripheral pattern in all the other lung diseases and in the few male normal controls with ANA probably differs from the 'shaggy' membrane pattern described by Casals, Friou and Teague, (1963) in SLE. Certainly, the peripheral pattern described in this study was more 'uniform' than 'shaggy', and more frequently related to IgM ANA than to other immunoglobulin classes. The peripheral pattern in active SLE usually relates to IgG ANA, complement fixing antibodies to DNA and especially high DNA binding levels (Rothfield and Stollar, 1967; Luciano and Rothfield, 1973). In our patients, complement fixing ANA was significantly rare in sera with a peripheral pattern, and the pattern was not seen in the few sera with positive DNA-binding.

The observation that a homogeneous pattern was the most common immunofluorescent staining pattern in 'pleuropulmonary' SLE and that it appears to relate to deoxyribonuclease sensitive antigens resembles the findings in other patients with SLE (Beck, 1963). It

was also the most common pattern in the other lung diseases.

One other feature common to 'pleuropulmonary' SLE and CFA and distinguishing them from the other lung diseases was the demonstration of nucleolar patterns of staining in a small number of patients. It was interesting that these patterns were associated with high titres of ANA, mixtures of IgG, IgM and IgA ANA's and complement fixing ANA. Miyawaki and Ritchie (1974) reported that the nucleolar ANA's in patients with systemic sclerosis or atypical illnesses associated with Raynaud's phenomenon nearly always occur in sera with more than two immunoglobulin classes of ANA, and frequently fix C3 and C4. Raynaud's phenomenon was observed in five of the patients with 'pleuropulmonary' SLE but none had nucleolar ANA, and scleroderma was diagnosed in three of the patients with CFA only one of whom had nucleolar staining.

The observation that nucleolar and speckled staining patterns in 'pleuropulmonary' SLE related to saline soluble or deoxyribonuclease sensitive antigens, but that similar patterns in CFA related to ribonuclease sensitive antigens suggests that different antibodies are involved. Speckled patterns in SLE have previously been described in association with a phosphate buffer extracted 'Sm' antigen (Tan and Kunkel, 1966) and with a protease sensitive, ribonuclease insensitive nuclear antigen (Sharp et al, 1972). In CFA it is possible that

speckled patterns may resemble those associated with a ribonuclease sensitive, protease insensitive antigen described by Sharp et al (1972) in mixed connective tissue disease.

Although there was little evidence of antibodies to ribonuclease sensitive antigens or of precipitins to depolymerized RNA in 'pleuropulmonary' SLE, nevertheless there have been several reports of antibodies to ribonucleate antigens in SLE. Precipitating antibodies to soluble ribonucleoprotein of cytoplasmic origin have been described (Reichlin and Mattioli, 1972; Mattioli and Reichlin, 1974), and precipitating antibodies to ribonucleoprotein of nuclear origin in SLE, scleroderma and mixed connective tissue diseases (Parker, 1973). Antibodies to double-stranded but not single-stranded RNA have been described in between 54% and 70% of patients with SLE and with a slightly lower frequency, 14% to 38% in other connective tissue disorders (Nassonova et al, 1972; Sylvester et al, 1973). They also occur spontaneously in NZB mice (Powell and Steinberg, 1972; Talal, 1973). The failure to demonstrate precipitins to low molecular weight RNA in this study is in keeping with recent observations which have indicated that the immunogenicity of RNA depends especially on the conformation of the double-helix rather than on the presence of a given base or the nature of the sugar (Nahon, Moulart and Lacour, 1973).

Lymphocyte Sensitization

These studies using the leucocyte migration test as an in vitro

correlate of cell-mediated immunity suggest that nuclear antigens can stimulate cellular as well as humoral immune responses in patients with 'pleuropulmonary' SLE, and moreover also in patients with CFA and asbestosis.

The numbers of patients included in the lymphocyte studies were too small to allow accurate conclusions with regard to prevalence, but well over half the patients with 'pleuropulmonary' SLE appear to show lymphocyte sensitization using whole rat liver nuclei, calf thymus deoxyribonucleoprotein and commercial calf thymus DNA. The evidence of lymphocyte sensitization to deoxyribonucleoprotein is in keeping with a previous report of delayed intracutaneous skin tests with homologous nucleoprotein in patients with SLE (Azoury, Jones and Gum, 1966) and is another feature distinguishing patients with 'pleuropulmonary' SLE from those with other lung diseases. The positive inhibition of leucocyte migration to commercial calf thymus DNA supports the previous evidence of lymphocyte sensitization to DNA in SLE obtained with a variety of methods. These have included blast transformation of lymphocytes (Patrucco, Rothfield and Hirschorn, 1967; Reinert et al, 1971) and delayed hypersensitivity skin reactions (Azoury, Jones and Gum, 1966; Reinert et al, 1971) as well as inhibition of peripheral leucocyte migration (Muller-Berat and Moulias, 1970; Moulias et al, 1970: Harpey et al, 1971; Galanaud et al, 1971). However, this study has demonstrated that lymphocyte sensitization to DNA is not unique to patients with SLE but also frequently

occurs in ANA positive patients with CFA and asbestosis.

The observation that, although antibodies to deoxyribonucleoprotein and DNA were present in many patients with 'pleuropulmonary' SLE, the antibody and lymphocyte responses rarely occurred together suggests that the positive inhibition is unlikely to be mediated by antibody. This is also supported by the lack of correlation between positive inhibition and the ANA titre. The interesting dissociation between antibody and lymphocyte responses may be a reflection of host differences.

In conclusion it appears that reactivity to deoxyribonucleoprotein in lung disease, either antibody production lymphocyte sensitization or both, is especially characteristic of patients with 'pleuropulmonary' SLE but that antibody and lymphocyte responses to DNA may occur in several lung diseases.

Peripheral leucocyte tests with whole rat liver nuclei were sometimes negative when positive responses were observed with purified antigens. This suggests that the whole nuclear preparation may contain insufficient amounts of antigens for lymphocyte sensitization to be detected in some instances. The patients who had circulating ANA but who did not show lymphocyte sensitization to any antigen may be either without lymphocyte involvement or may be sensitized to other types of nuclear antigens not tested here in purified form and not present

in sufficient quantities in the crude nuclear preparation.

These findings suggest that lymphocyte-mediated hypersensitivity reactions may contribute to the tissue damage in some patients with 'pleuropulmonary' SLE. The complexity of the clinical situation, as well as the complex immunological findings has, however, confused attempts to determine whether any particular clinical presentation is related to any distinctive immunological observation.

Possible Stimulatory Pathways

The reasons for initiation and maintained stimulation of multiple immune responses to nuclear antigens in patients with SLE are unknown but certain observations allow speculation. There is some evidence of persistent virus in patients with SLE (Klippel et al, 1974; Metz and Metz, 1974: Moolten, 1974; Schwartz, 1975; Zhdanov, 1975) and it seems possible that responses to deoxyribonucleoprotein may be stimulated by viral nucleoprotein or as a consequence of viral induced changes in host nucleoprotein. Responses to other nuclear antigens might be stimulated by other viral presentations, or by host antigens released from damaged cells. In the drug-induced lupus syndromes there is evidence that some drugs combine with host nucleoproteins (Tan, 1968a; Alarcon-Segovia, 1973) suggesting that this type of altered host antigen might achieve by-pass of tolerance. It is also of interest, in view of the photosensitivity of many patients with SLE, that ultra-violet light denatured DNA can stimulate antibodies to DNA in experimental animals (Tan, 1968b). Ultra-violet light irradiation is also a method of inducing replication of latent virus (Davis et al, 1968)

should virus prove important as an antigenic stimulus in SLE. Family studies (Pollak, Mandema and Kark, 1960; Chantler et al, 1973) and studies in NZB/NZW hybrid mice (Braverman and Marino, 1966) have suggested that SLE may be genetically determined. There is also some evidence of abnormality in the T-lymphocyte population in patients with SLE (Horwitz, 1972; Hahn, Bagby and Osterland, 1973). These last observations have posed the question of defective tolerance control.

Whatever the sources of stimulatory nuclear antigens in patients with SLE, reasons must also be found for maintained antigenic stimulation to explain the persistent responses. Biochemical abnormalities which might lead to prolonged maintenance of some types of nuclear antigens in the circulation are increased levels of deoxyribonuclease inhibitor in patients with SLE and ANA positive patients with other diseases (Frost and Lachmann, 1968) and in NZB/NZW hybrid mice (Hadjiyannaki and Lachmann, 1972), and a slow rate of drug acetylation in drug-induced lupus and in the spontaneous form of the disease (Alarcon-Segovia, Fishbein and Alcala, 1971; Godeau et al, 1973).

Main Conclusions

Patients with 'pleuropulmonary' SLE resemble those referred to other specialists having multiple responses which may involve different nuclear antigens, several immunoglobulin classes of antibodies and sensitized lymphocytes even in individual patients. The variety of

responses is greater than in any other lung disease. Distinctions from other groups of patients with SLE are the low frequency of antibodies binding with double-stranded DNA and of the 'shaggy' membrance pattern of immunofluorescent staining, which suggest a sub-acute form of the disease.

Deoxyribonucleoprotein appears to be an important antigen stimulating antibody production in the majority of patients, and in some cases lymphocyte sensitization. It was very rarely involved in the other lung diseases. DNA also appears important. Precipitating antibodies were detected in about half the patients and lymphocyte sensitization in others, however similar responses to DNA were demonstrated in other lung diseases. It is suggested that single stranded DNA may be the antigenic stimulus in most instances except in a small number of patients who were distinct in having antibodies binding with double-stranded DNA.

The identification of complement fixing ANA in just under half the patients suggests antibodies with the potential to mediate tissue-damaging reactions. The evidence of lymphocyte sensitization to deoxyribonucleoprotein and DNA suggests also a possibility of lymphocyte-mediated hypersensitivity reactions.

Reasons for variable responses to nuclear antigens in patients with 'pleuropulmonary' SLE are not known but may reflect a range of

stimulatory mechanisms. The absence of relationship between antibody and lymphocyte responses to the same antigen suggests that host differences may determine some of the immunological differences. Another theory is that persistent virus might explain a multiple antigenic stimulation including the unusual reactivity to deoxyribonucleoprotein.

These observations, when compared with the findings in the other lung diseases, not only support the proposal that immune responses to nuclear antigens may arise in different ways in different lung diseases but also suggest that even in 'pleuropulmonary' SLE they may arise in different ways.

SENSITIZATION TO NUCLEAR ANTIGENS IN CRYPTOGENIC FIBROSING ALVEOLITIS

Antinuclear Antibodies

Unlike 'pleuropulmonary' SLE, not all patients with CFA had ANA immunofluorescence. The prevalence (37%) was similar to that in silicosis (44%) but higher than in asbestosis (26%) and intrinsic asthma (27%). The range of ANA titres also resembled that in silicosis, lower than in 'pleuropulmonary' SLE, but higher than in asbestosis. Interestingly, males with CFA had a lower prevalence and lower range of ANA titres than females with CFA, and in these respects resembled the male asbestosis group.

ANA production in CFA, as in 'pleuropulmonary' SLE did not appear to be a consequence of aging occurring similarly in young, under 40

years, and older, over 40 years, age groups. Since ANA was not detected in normal healthy controls of under 40 years of age in this or other normal control studies, the presence of ANA in younger patients indicates that ANA production is likely to be linked with the disease process.

The ANA's in CFA were distinct from those in 'pleuropulmonary' SLE but resembled those in other ANA positive lung diseases generally unable to react with deoxyribonucleoprotein, induce LE cell formation, or react with DS-DNA in Farr-binding tests. This suggests differences in the stimulatory antigens.

Complement fixing ANA

A most interesting finding was that CFA resembled 'pleuropulmonary' SLE but was distinct from the other lung diseases in having complement fixing ANA in just over a third of the ANA positive patients. Thus in CFA, as well as in 'pleuropulmonary' SLE, at least some of the ANA's have the potential to take part in tissue damaging reactions.

SS-DNA precipitins

The observation that in CFA as in 'pleuropulmonary' SLE complement fixing ANA tended to occur in sera with precipitins to SS-DNA suggests that some precipitating antibodies to SS-DNA in patients with CFA may fix complement. However, since many other patients had SS-DNA precipitins without complement fixing ANA there is again a possibility

of different types of antibodies reacting with SS-DNA. There may perhaps be slightly different forms of stimulatory antigens, or alternatively host determined differences of immunoglobulin subclass response.

Inability to fix complement does not necessarily mean that an antibody cannot take part in tissue damaging reactions, other possible routes include those involving reaginic antibodies (Coombs and Gell, 1975), non-specific stimulation of T-lymphocytes by immune complexes (Moller, 1969) or antibody dependent 'K-cell' cytotoxicity (MacLennan, 1972).

A suggestion of a generally higher frequency of complement fixing ANA in female patients over males, and a trend towards a higher frequency of SS-DNA precipitins in females over males emphasizes that caution should be given at this stage to a definite conclusion that 'pleuropulmonary' SLE and CFA are distinct from asbestosis and silicosis in possessing complement fixing ANA in association with precipitins to SS-DNA, since the patients with the industrial lung diseases were mostly male.

In other studies (Turner-Warwick, Haslam and Weeks, 1971) we have been unable to obtain convincing evidence that immune complexes play any important role in the pathogenesis of CFA. Immunofluorescent examination of biopsy and /or post mortem lung material from thirty- three patients demonstrated evidence of complement fixing immune-complexes in only six instances, but since CFA is probably a syndrome of mixed etiology (Turner-Warwick, 1972) it was significant that all six patients had circulating ANA. Serum from three of the six

was available for inclusion in the present study. Two had precipitins to SS-DNA together with complement fixing ANA, and both had IgG ANA. The remaining patient had IgM ANA and precipitins reacting with 'DS' but not SS-DNA, but did not have circulating complement fixing ANA. None of the patients reacted with deoxyribonucleoprotein or with DS-DNA in Farr-binding tests, but one with IgG ANA had positive LE cells and clinical features of systemic disease suggesting an overlap with SLE.

DS-DNA precipitins

The close correlation of the precipitin results with 'DS' and SS-DNA in CFA suggests that in most instances SS-DNA may be the reactive antigen. The small number of sera precipitating only with 'DS'-DNA have not yet been re-tested after absorption with SS-DNA but none showed positive binding with DS-DNA. There is thus no convincing evidence from these studies of sensitization to DS-DNA in CFA.

Immunoglobulin classes of ANA

Certain patients with CFA and 'pleuropulmonary' SLE had mixtures of IgG, IgM and IgA ANA's. These triple mixtures were rare in patients with other lung diseases. The patients also tended to have high titres of ANA and complement fixing ANA. There was however, no especial

tendency for such patients with CFA to have a systemic disorder. Another feature which distinguished CFA from 'pleuropulmonary' SLE was the presence of lone IgM ANA in some patients.

Immunofluorescent staining patterns

While the homogeneous immunofluorescent staining pattern was the most common pattern in CFA as in the other lung diseases, the frequent demonstration of the peripheral pattern again conflicts with the findings in 'pleuropulmonary' SLE. As previously discussed (p312) this peripheral pattern associated with IgM ANA is probably distinct from the 'shaggy' membrane pattern associated with IgG ANA which has been described in some patients with SLE (Casals, Friou and Teague, 1963). The possible distinction between nucleolar and speckled staining patterns in CFA and 'pleuropulmonary' SLE has also already been discussed (p.315) together with evidence that these patterns may relate to ribonucleate antigens in CFA. Two of the patients with CFA and speckled staining had evidence of a mixed connective tissue disorder, but many patients with speckled or nucleolar patterns had only the lung disease. The demonstration of antibodies to ribonuclease sensitive antigens in CFA is of interest since in an earlier study we found complement fixing antibodies to cytoplasmic tissue components in 27.5% of patients with CFA (Turner-Warwick and Haslam, 1971). These were reacting mainly with cytoplasmic ribosomes. This suggests that ribonucleoprotein of cytoplasmic as well as nuclear ribosomes may be involved in antibody activity in CFA, and it may be

worthwhile investigating reactivity to ribonucleoprotein in CFA in greater detail. Antiribosomal antibodies have been described in up to 29% of patients with SLE (Schur, Moroz and Kunkel, 1968).

Deoxyribonucleoprotein and DS-DNA binding antibodies

The six patients with CFA who had positive reactions with deoxyribonucleoprotein remarkably were the rare patients with associated idiopathic liver disease. Only one had LE cells and in addition to lung and liver involvement, this patient had other systemic changes suggestive of SLE. This observation raises the question of viral etiology for the liver disease and also the question of whether the lung disease in these patients is related to the same etiologic agent as the liver disease.

Just three patients with CFA had shown positive Farr-binding with DS-DNA, and these did not react with deoxyribonucleoprotein nor did they clinically fulfil the criteria for SLE. However, all did have systemic changes in addition to the lung disease, one with scleroderma; one with liver disease and the third with rheumatoid arthritis and vasculitis.

It is possible that these immunological parameters may help to identify patients with CFA who have systemic disease, a rapid progression and poor prognosis.

Lymphocyte Sensitization

This study has shown for the first time that commercial calf thymus DNA inhibits the migration of leucocytes from just under half of untreated patients with CFA. The lack of correlation with the antibody results using the same antigen suggests that, like certain patients with 'pleuropulmonary' SLE, these patients may mount a cellular immune response to this antigen. However, in CFA there was no evidence of lymphocyte sensitization to deoxyribonucleoprotein. This is in keeping with the antibody findings and provides further evidence of different stimulatory mechanisms in CFA and 'pleuropulmonary' SLE. This has relevance to the clinical situation since it suggests that patients with CFA who respond to nuclear antigens do not necessarily fall into a subgroup with a clinical and possibly an etiological overlap with SLE.

The positive response to SS-DNA in a patient who had also reacted with the unheated 'DS'-DNA suggests that, as in the antibody studies, the reactive antigen may in most instances be SS-DNA in the damaged commercial 'DS'-DNA. Patients who did not show lymphocyte sensitization to calf thymus DNA might still be sensitized to other types of nuclear antigen since the whole rat liver nuclei appeared an unsatisfactory screening antigen. Cell-mediated immune hypersensitivity reactions triggered by lymphocytes sensitized to DNA may, therefore, contribute to tissue damage in a number of ANA positive patients with CFA. The real contribution of lymphocyte-mediated hypersensitivity reactions to tissue damage in CFA is, however, difficult to assess using methods such as peripheral leucocyte migration which require a fairly pure form of an identified antigen.

In CFA there is no known extrinsic etiologic agent (Turner-Warwick, 1972). There are several different kinds of anti-tissue reactivity but the antigens involved even in antinuclear reactivity have still to be properly identified (Turner-Warwick and Haslam, 1971). What is required is a method to look for evidence of lymphocyte-mediated hypersensitivity which is not dependent upon antigen. In an earlier unpublished study, we obtained evidence that circulating lymphocytes from patients with CFA could be directly cytoxic to Wistar strain Wl-38 human lung fibroblast tissue culture monolayer target cells. More attention should be focussed on this type of study which is particularly valuable since it not only serves as a marker of possible lymphocyte sensitization but also confirms that these are primed for tissue-damage. Many other methods merely confirm that lymphocyte sensitization has at some time occurred.

Possible Stimulatory Pathways

Nothing is known of the pathways leading to stimulation of immune responses to nuclear and other tissue antigens in CFA. Family studies (Peabody et al, 1950; Bonanni, Frymoyer and Jacox, 1965; Swaye et al, 1969) and recent evidence of an increased prevalence of HLA W12 in patients with CFA (Evans, C.C., personal communication) have suggested some element of genetic predisposition for the disease. It is, therefore, possible that there is a genetic predisposition for anti-tissue reactivity.

Viral etiology has been suggested (Golden and Tullis, 1949; Pokorney and Hellwig, 1955) but with only minimal supportive evidence (Webster, 1972). If confirmed this might also provide explanations for the anti-tissue responses.

The main finding of precipitating antibodies to SS-DNA but not to DS-DNA in CFA resembles the findings in connective tissue disorders other than SLE, for example rheumatoid arthritis and systemic sclerosis (Koffler et al, 1971; Hughes, 1975) which can also occur in clinical association with CFA. This may reflect a similar mode of sensitization to nuclear antigens in these disorders.

Main Conclusions

Cryptogenic fibrosing alveolitis is distinct from all the other lung diseases except 'pleuropulmonary' SLE in having complement fixing ANA together with precipitins to SS-DNA in just over a third of the ANA positive patients. However, it is distinguished from 'pleuropulmonary' SLE by the virtual absence of LE cells, antibodies reacting with deoxyribonucleoprotein and of antibodies binding with DS-DNA. It resembles silicosis, asbestosis and intrinsic asthma in having some patients with precipitins to SS-DNA but without complement fixing ANA. The prevalence and range of ANA titres in CFA is similar to silicosis, lower than in pleuropulmonary SLE but higher than in the other diseases, and in fact over half the patients have negative nuclear immunofluorescence.

CFA is a syndrome of probable mixed etiology, and the immunological

findings appear to demonstrate a number of clinical subgroups. The few patients showing reactivity to deoxyribonucleoprotein all have associated liver disease possibly suggesting viral etiology, and the few with positive Farr-binding to DS-DNA have systemic disease rather than lone lung involvement.

The nuclear antigens stimulating the responses in the majority of these patients with CFA remain unknown. However, the different immunoglobulin classes of ANA and different patterns of ANA immunofluorescence suggest the involvement of several different antigens. It is felt that reactivity to ribonucleoprotein of nuclear and cytoplasmic origin should be investigated further.

Evidence of lymphocyte sensitization probably to SS-DNA suggests that this may trigger tissue damaging cell-mediated hypersensitivity reactions in some patients with CFA. It is also possible that this antigen may be involved in tissue damage via immune complex formation with complement fixing antibody.

The findings in the different lung diseases favour the proposal that different responses are stimulated according to the clinical circumstances, but it must be explained why this is shown by different combinations of features with some overlap between the diseases rather than by distinct differences of response. The common production of complement fixing ANA in CFA and 'pleuropulmonary' SLE may reflect some similarity of host responsiveness but the other

observations suggest a different kind of antigenic stimulation. The overlap between the diseases in responding to SS-DNA does not necessarily indicate a common stimulatory mechanism. There are several possible antigenic determinants on SS-DNA, and antibodies have been described reacting with different base determinants (Lacour, Nahon-Merlin and Michelson, 1973).

SENSITIZATION TO NUCLEAR ANTIGENS IN ASBESTOSIS

Antinuclear Antibodies

The prevalence of ANA in asbestosis was higher than in age and sex matched normal controls, but lower than in 'pleuropulmonary' SLE, CFA and silicosis. In addition, the ANA titres were lower than in any other group except males with CFA. In contrast with the ANA's of 'pleuropulmonary' SLE, none of those in asbestosis were able to induce LE cell formation, react with deoxyribonucleoprotein or bind with DS-DNA. Nor were there any able to fix complement as in 'pleuropulmonary' SLE and CFA. Ouchterlony precipitins to SS-DNA were present in several sera, and resembled the precipitins to SS-DNA in some patients with CFA, silicosis and intrinsic asthma which are not associated with complement fixing ANA. It has already been suggested that there may be several types of antibodies to SS-DNA relating to different antigenic determinants.

The features which particularly characterized the ANA's of asbestosis were a higher prevalence of IgM ANA and a lower prevalence of IgG ANA than in males with any other lung disease, and especially characteristic was the large number of sera with lone IgM ANA. This suggests that fairly limited pathways lead to ANA production in asbestosis. Asbestosis was the only lung disease where there was evidence of a link between aging and ANA production. It was the

only disease where the prevalence of ANA increased with increasing patient age, and where only in the older age groups was the prevalence significantly above that in age matched normal controls. Additionally interesting was the general finding that patients with lone IgM ANA were in an older age range than those with other immunoglobulin class profiles. If there is any similarity between the low titre IgM ANA's of asbestosis and the age-related low titre IgM ANA's of normal controls the results of this study suggest that in asbestosis a prevalence of ANA is achieved over one decade which under normal circumstances would take two decades. The impression gained is that asbestos dust might accelerate age-related ANA production. Asbestos dust irritation does not, however, by itself appear able to induce ANA production since the prevalence of ANA was not increased in a group of disease-free asbestos workers matched for sex, age and duration of exposure to asbestos dust with the asbestosis disease group.

Some element of genetic predisposition to develop asbestosis is suspected from individual variation in host susceptibility to the disease (Becklake et al, 1972) and the recent suggestion of a slightly increased prevalence of histocompatibility locus antigen 27 (HLA 27) in patients with asbestosis (Merchant et al, 1975). This latter study did not expose any association between HLA type and ANA or rheumatoid factor production, but it is still possible that genotypes predisposed to develop asbestosis may include those predisposed to produce tissue antibodies with age. It would then be

possible to speculate that asbestos dust may accelerate the expression of this predisposition for ANA production. If it should be confirmed that patients with ANA tend to develop the most severe forms of asbestosis (Soutar et al, 1974) this would suggest that a predisposition to produce ANA may also be a marker of a population especially susceptible to the disease, or alternatively that ANA may actively aggravate the development of the disease. However, studies of biopsy and post mortem lungs using immunofluorescent methods (Turner-Warwick, Haslam and Weeks, 1971) have failed to find any evidence of antibody contributing to the tissue damage in asbestosis. The inability of the ANA's in asbestosis to fix complement also suggests that they may be less likely to play an aggressive role than the complement fixing ANA's detected in 'pleuropulmonary' SLE and CFA.

The finding that SS-DNA was the only antigen identified contributing to ANA activity in asbestosis was of interest in view of the evidence that in mice aging is associated with an abnormal persistence of increasing amounts of nuclear DNA in single-stranded form (Chetsanga et al, 1975). The fibrogenic effect of asbestos dust is connected with enhanced intra-alveolar macrophage activity. (Pernis et al, 1966; Davis, 1967; Vigliani, 1968) which persists relentlessly because the inert nature of asbestos dust renders it resistant to macrophage attempts to degrade it. The resultant inflammatory response might cause the continued release of nuclear antigens from damaged intra-alveolar macrophages or other particularly vulnerable

cell types in the locality. If aging is associated with an abnormal build up of nuclear DNA in single-stranded form in humans as well as mice, the inflammatory response might then lead to a greater than normal amount of SS-DNA in the circulation and a premature stimulation of ANA in individuals with such a predisposition.

Lymphocyte Sensitization

The results of the lymphocyte studies in asbestosis were very similar to those in CFA, with just under half the ANA positive patients having evidence of lymphocyte sensitization to commercial calf thymus DNA, but very rarely to deoxyribonucleoprotein. Again it is possible that the reactive antigen is single-stranded DNA present in the damaged commercial material, but this must now be confirmed. In asbestosis, as in CFA and 'pleuropulmonary' SLE the dissociation between the antibody and lymphocyte findings provides evidence that the inhibition of leucocyte migration to DNA was not mediated by antibody. It therefore appears that SS-DNA is a fairly important antigen stimulating both lymphocyte and antibody responses in patients with asbestosis. There may be defective production of other nuclear antigens as well as DNA with age, and the patients who had circulating ANA but who did not react with DNA in the lymphocyte or antibody studies may be sensitized to such antigens.

This is the first time that evidence has been obtained that lymphocyte-mediated hypersensitivity reactions might contribute to the tissue damage in certain patients with asbestosis. It is also the only evidence in support of the idea that immune responses to nuclear

antigens might directly aggravate the disease. If immune hypersensitivity reactions prove unimportant, the responses may be harmless genotypic markers of a population especially susceptible to the disease.

Main Conclusions

The ANA's in asbestosis are distinct from those in other diseases since they are generally lone IgM ANA's of low titre. This and evidence that ANA production is associated with aging suggests a resemblance to the age-related ANA's of normal controls. Precipitins to SS-DNA are the only main group of antibodies yet identified in asbestosis.

There is no convincing evidence that ANA's play any role in tissue damage in asbestosis, but evidence of lymphocyte sensitization to commercial calf thymus DNA suggests that lymphocyte-mediated hypersensitivity reactions may aggravate tissue damage in certain patients.

A theory proposed for the source of stimulatory antigen is that this may be SS-DNA derived from damaged alveolar macrophages or other cells in the vicinity of inhaled dust particles at a time when aging may cause a persistence of intracellular DNA in single-stranded form. It is also suggested that defective nuclear antigens of other types may appear with age and explain the responses in other patients. The observations in asbestosis suggest that the pathways of sensitization may be limited in comparison with CFA and 'pleuropulmonary' SLE.

SENSITIZATION TO NUCLEAR ANTIGENS IN SILICOSIS

Silicosis was distinct from the other lung diseases in having a majority of ANA positive patients with lone IgG ANA and a lower proportion of IgM ANA than any other disease. The general features otherwise were a prevalence of ANA higher than in asbestosis, resembling CFA, and ANA titres generally higher than in asbestosis also resembling CFA but lower than in 'pleuropulmonary' SLE. Immunofluorescent patterns were either homogeneous or peripheral, and the ANA's were generally non-complement fixing, non-reactive with deoxyribonucleoprotein, unable to induce LE cell formation and negative in Farr binding tests with DS-DNA. However, as in the other diseases, many sera had precipitins to SS-DNA. The observation that slightly more sera were positive in precipitin tests with 'DS' than SS-DNA may be due to technical variation or may suggest that some patients have precipitins to double-stranded DNA.

Although both silicosis and asbestosis are fibrotic lung diseases associated with inorganic dust inhalation with a similar tendency to produce lone immunoglobulin classes of ANA and precipitins to SS-DNA, the immunoglobulin class difference suggests different pathways of ANA production. Since SS-DNA was of possible importance as a stimulatory antigen in both diseases there may be a slightly different form of denatured DNA antigen in the two diseases. Experiments in mice have shown that different immunoglobulin classes of DNA antibody can be

induced by presenting denatured DNA antigen in different ways. An IgM response is obtained using a complex of DNA with human serum albumin (Sandberg and Stollar, 1966) and an IgG response using DNA-methylated bovine serum albumin complexes (Stollar and Sandberg, 1966).

The theory proposed for the source of SS-DNA antigen in asbestosis is an unlikely explanation in silicosis since ANA production was not associated with aging. In silicosis as well as asbestosis macrophages are important in the response to dust inhalation, but unlike inert asbestos ores silica salts are highly reactive chemically and the phagocytosed dust is highly toxic (Vigliani Pernis and Monaco, 1961; Allison, Harington and Birbeck, 1966; Allison, 1968). Fibrogenesis is particularly dramatic and appears to be associated with rapid and extensive macrophage killing with release of fibrogenic stimulating factors (Heppleston and Styles, 1967; Heppleston, 1969). In theory ANA production in silicosis may be a response to intracellular antigens including DNA which are altered perhaps by chemical interaction with silica or enzymes from damaged phagosomes and then released after macrophage death. The adjuvant effect of silica (Pernis and Paronetto, 1962) may also aid overthrow of tolerance.

A recent radiographic study has suggested that patients with silicosis who have ANA may develop particularly severe disease (Jones et

al, 1976). It is not, however, known whether the ANA's take part in tissue damaging reactions which aggravate the disease. The infrequency of complement fixing ANA suggests that Type III hypersensitivity reactions involving nuclear antigens are unlikely to be important.

In view of the occasional case reports of silicosis associated with SLE the LE cells and positive Farr binding with DS-DNA in one patient and the positive reactivity to deoxyribonucleoprotein in another was of interest. However, neither had clinical evidence of SLE.

It is not yet known whether nuclear antigens stimulate lymphocyte sensitization in silicosis since it was not possible to obtain lymphocytes from these patients for inclusion in the present study.

Main Conclusions

Silicosis is distinct from the other lung diseases in having a majority of ANA positive sera with lone IgG ANA. Resembling asbestosis, precipitins to SS-DNA are the only main type of antibodies identified. However, ANA production in silicosis, unlike asbestosis, does not appear to be a consequence of aging. It is suggested that host intracellular antigens altered by chemical interaction with silica or phagosomal enzymes and released from intra-alveolar macrophages killed after phagocytosis of toxic silica

dust, may be a source of antigen(s) stimulating ANA production in silicosis. It is also suggested that antigenic stimulation may be aided by the adjuvant effect of silica.

It is not yet known whether the ANA's in silicosis can take part in tissue damaging reactions, nor whether nuclear antigens can stimulate lymphocyte sensitization as well as antibody production.

SENSITIZATION TO NUCLEAR ANTIGENS IN INTRINSIC ASTHMA

Discussion of the ANA's in this disease is less satisfactory because of the small number of patients studied. There was just one interesting feature, the frequent finding of mixtures of IgG with IgM ANA. The only other disease where sera frequently contained this mixture was 'pleuropulmonary' SLE. There was, however, no general resemblance between the ANA's in intrinsic asthma and those in 'pleuropulmonary' SLE. Only one patient had antibodies to deoxyribonucleoprotein and none had LE cell factor, positive Farr binding with DS-DNA or complement fixing ANA, and even precipitins to SS-DNA were apparently less common than in the other diseases. It is nevertheless possible that the mixtures of IgG and IgM ANA's may reflect some immunological link between the two diseases. A theory that they may share an ability to produce reaginic ANA has already been discussed (p.311).

There was no evidence of lymphocyte sensitization to deoxyribonucleoprotein, DNA or whole nuclei in this small group of untreated ANA positive patients with intrinsic asthma. Additional tests using SS-DNA and depolymerized RNA in four patients were also negative. Practically nothing is known of the etiologic agent(s) in intrinsic asthma, and the generally negative findings in this study give no guide to the identity of the antigens stimulating ANA production. The fact that the ANA's were easily detected and of reasonably high titre in most of these patients suggests that the generally negative antibody and lymphocyte findings in this group are not due to low levels of sensitization. It is more likely that the main antigen stimulating ANA in intrinsic asthma is of a type which has not yet been examined. If an antigen is identified, it will then be possible to look again for evidence of lymphocyte involvement.

THE INFLUENCE OF CORTICOSTEROID THERAPY

The few patients with 'pleuropulmonary' SLE and CFA examined in lymphocyte tests before and then again during corticosteroid therapy brought to light the very marked influence of corticosteroid therapy in reducing inhibition of leucocyte migration. Corticosteroids may directly inhibit T-lymphocyte responses for example by inhibiting

lymphokine release (Eaglstein, Reasor and Hsia, 1969), or they may interfere with the vivo inflammatory reaction limiting the availability of sensitized lymphocytes (Ernström and Larsson, 1967). Whatever the explanation, the importance of appreciating the considerable influence of corticosteroid therapy on leucocyte migration is emphasized.

We have not yet satisfactorily examined the influence of corticosteroid therapy on antibody production to nuclear antigens in our patients with 'pleuropulmonary' SLE and CFA. While we have previously shown that neither the presence or titres of immunofluorescent ANA are perceptibly affected by corticosteroid therapy, we have not looked closely at the different types of nuclear antibodies. Case reports of patients with SLE have demonstrated that antibodies to DNA can fluctuate quite markedly monitoring periods of acute disease and therapeutically induced remissions (Schur and Sandson, 1968; Lightfoot, Redecha and Levsanos, 1975). There were just two patients with 'pleuropulmonary' SLE in the lymphocyte studies who were tested before and during corticosteroid therapy who happened to have precipitating antibodies to calf thymus DNA. Interestingly, the precipitin line in one of these patients initially very strong, was reduced to a mere trace after corticosteroid therapy. The ANA titres on the other hand did not change. Neither patient had antibodies to deoxyribonucleoprotein. The three patients with CFA tested before and during corticosteroid therapy unfortunately did not

have antibodies either to DNA or to deoxyribonucleoprotein. Again, the ANA titres were unaffected by corticosteroid therapy. In this study the only group which included some patients receiving corticosteroid therapy was CFA, and the possibility that this might have influenced the results of tests for certain kinds of antibodies should therefore be borne in mind.

SUMMARIZED CONCLUSION

1. This study has confirmed that the prevalence of immunofluorescent ANA in random normal controls is very low but increases with increasing age.

2. It has also been confirmed that there is an increased prevalence of immunofluorescent ANA in certain lung diseases which cannot be explained as a simple consequence of variation in patient age or sex.

3. It has been shown for the first time that DNA may stimulate cellular as well as antibody responses in patients with CFA and asbestosis, and further evidence has been obtained that DNA and deoxyribonucleoprotein may stimulate cellular as well as antibody responses in patients with SLE.

4. Differences have been demonstrated in the titres as well as the prevalence of positive immunofluorescent ANA in different lung diseases which may suggest host differences or variable antigenic challenge.

5. Differences have been demonstrated in the numbers and types of immunoglobulin classes of ANA in the different lung diseases perhaps a consequence of different stimulatory antigens or a different time course of antigenic stimulation. Multiple

immunoglobulin classes of ANA were frequent in sera from patients with SLE, CFA and intrinsic asthma, but lone IgM ANA was the most common finding in asbestosis and lone IgG ANA in silicosis.

6. Differences have been demonstrated in the complement fixing ability of the ANAs in different lung diseases. ANAs able to fix complement were detected in a third of the ANA positive patients with SLE and CFA but very rarely in other diseases. It is not known whether this relates to a particular type of antigenic stimulation or to immunoglobulin subclass differences.

7. Differences in the identity of the stimulatory antigens in the different lung diseases have been suggested. Responses to deoxyribonucleoprotein were common in SLE but were otherwise rare except in a few patients with CFA and associated idiopathic liver disease. Farr assays suggested the possible involvement of double-stranded DNA in some patients with SLE. Single-stranded DNA appeared a fairly common antigen in all the diseases except intrinsic asthma. It is suggested that the form of denatured DNA antigen may differ in different diseases. Studies of speckled and nucleolar staining patterns in a small number of patients with SLE and CFA suggested that these relate to saline soluble or deoxyribonuclease-sensitive antigens in SLE but to ribonuclease-sensitive antigens in CFA.

8. These distinctive types of responses to nuclear antigens in different lung diseases support the thesis that the responses arise in different ways dependent upon the clinical circumstances. The alternative proposal that they arise as a common consequence of extensive tissue destruction in the lung seems unlikely since the only differences then expected would perhaps be quantitative in accordance with the degree of tissue destruction.

9. Individual patient differences within diseases, for example a dissociation of antibody and lymphocyte responses to the same antigen, suggest that some of the observed variability of response may be determined by individual differences of immune disposition.

10. The antigenic stimuli in the different lung diseases remain unknown but the observations in SLE suggest a multiplicity of stimulatory antigens triggering different facets of the immune system. It is proposed that persistent virus might provide one explanation for a multiple antigenic stimulation. In CFA the observations also suggest multiple stimulation. The observations in asbestosis and silicosis suggest that the stimulatory pathways are more limited than in SLE and CFA. It is proposed that SS-DNA derived from dust-damaged intra-alveolar macrophages at a time when ageing may cause a persistence of intracellular DNA

in single-stranded form may be a stimulatory antigen in asbestosis. In silicosis it is proposed that host antigens altered by chemical interaction with silica salts then released from dead intra-alveolar macrophages may stimulate the responses. The observations in intrinsic asthma give no guide to the initiating antigen.

11. As a result of this study it may now be proposed that different responses to nuclear antigens in different lung diseases may have variable biological properties which confer a variable ability to take part in tissue damaging reactions. The complement fixing ANA in SLE and CFA suggests a possibility of antibody-mediated tissue damaging hypersensitivity reactions in some patients. The evidence of lymphocyte sensitization to DNA and deoxyribonucleoprotein in SLE and to DNA in CFA and asbestosis suggests also a possibility of lymphocyte-mediated tissue damaging hypersensitivity reactions.

BIBLIOGRAPHY

AARDEN, L.A., De GROOT, E.R. & FELTKAMP, T.E.W. (1975)
Immunology of DNA 111. Crithidia Luciliae: A simple substrate for the detection of anti-DS DNA with the immunofluorescence technique.
Ann. N.Y. Acad. Sci. 254, 505.

ABLIN, R.J. (1972)
Malignancy associated with antinuclear antibodies.
Lancet 2, 1253.

AGNELLO, V., CARR, R.I., KOFFLER, D. et al. (1969)
Gel diffusion reactions of C1q with aggregated gamma globulin, DNA and various other anionic substances.
Fed. Proc. 28, 696.

ALARCON-SEGOVIA, D. (1969)
Drug-induced lupus syndromes.
Mayo Clin. Proc. 44, 664.

ALARCÓN-SEGOVIA, D. (1973)
Isoniazid, antinuclear antibodies and SLE.
Chest. 63, 299.

ALARCÓN-SEGOVIA, D., FISHBEIN, E., ALCALÁ, H. et al. (1970)
The range and specificity of antinuclear antibodies in systemic lupus erythematosus.
Clin. Exp. Immunol. 6, 557.

ALARCÓN-SEGOVIA, D., FISHBEIN, E. & ALCALÁ, H. (1971)
Isoniazid acetylation rate and development of antinuclear antibodies upon isoniazid treatment.
Arthritis Rheum. 14, 748.

ALEXANDER, W.R.M., BREMNER, J.M. & Duthie, J.J.R. (1960)
Incidence of antinuclear factor in human sera.
Ann. Rheum. Dis. 19, 338.

ALLISON, A.C. (1968)
Lysosomes and the responses of cells to toxic materials.
Sci. Basis Med. Ann. Rev. p.18.

ALLISON, A.C. (1973)
Mechanisms of tolerance and autoimmunity.
Ann. Rheum. Dis. 32, 283.

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971)
Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity.
Lancet 2, 135.

ALLISON, A.C., HARINGTON, J.S. & BIRBECK, M. (1966)
An examination of the cytotoxic effects of silica on macrophages.
J. Exp. Med. 124, 141.

AMBROSI, L. (1966)
Relationships between pulmonary and extrapulmonary histological changes in silicosis. Pathogenetic considerations.
Med. Lav. 57, 249.

ANDO, T. (1966)
A nuclease specific for heat-denatured DNA isolated from a product of Aspergillus oryzae.
Biochim. Biophys. Acta 114, 158.

ARANA, R. & SELIGMANN, M. (1967)
Antibodies to native and denatured deoxyribonucleic acid in systemic lupus erythematosus.
J. Clin. Invest. 46, 1867.

ARTHRITIS AND RHEUMATISM COUNCIL WORKSHOP (1976)
Anti-DNA antibodies.

AZOURY, F.J., JONES, H.E. & GUM, O.B. (1966)
Antinuclear factors and intradermal tests in systemic lupus erythematosus.
Fed. Proc. 25, 680.

BARBU, Z., SZABO, S., LUKACS, E. et al. (1969)
Antilung autoantibodies in workmen exposed to silicogen dust.
Ftiziologia 18, 315.

BARNETT, E.V., CONDEMI, J.J., LEDDY, J.P. et al. (1964)
Gamma $_2$, gamma $_{1A}$ and gamma $_{1M}$ antinuclear factors in human sera.
J. Clin. Invest. 43, 1104.

BARNETT, E.V. & ROTHFIELD, N. (1969)
Arthritis. Rheum. 12, 543. The present status of antinuclear antibody serology

BAUM, J. & ZIFF, M. (1962)
7S and macroglobulin antinuclear fluorescence factors in systemic lupus erythematosus and rheumatoid arthritis (abstract)
Arthritis Rheum. 5, 636.

BECK, J.S. (1963)
Auto antibodies to cell nuclei.
Scott. Med. J. 8, 373.

BECK, J.S. & HUGHES, P. (1970)
In-vivo nuclear localisation of human antinuclear antibodies in mice with carbon tetrachloride and thioacetamide-induced hepatic necrosis.
J. Pathol. 101, 11.

BECKLAKE, M.R., FOURNIER-MASSEY, G., ROSSITER, C.E. et al. (1972)
Lung function in crysotile asbestos mine and mill workers of Quebec.
Arch. Environ. Health 24, 401.

BELLI, E. (1969)
Tubercular toxins as asthma antigens.
Minerva Med. 60, 4345.

BENNICH, H., BERGQUIST, N.R., BERGSTRAND, A. et al. (1974)
Failure to demonstrate immunoglobulin E in glomeruli of patients with various nephropathies.
Acta Pathol. Microbiol. Scand. /B/ 82, 245.

BLOMGREN, S.E., CONDEMI, J.J. & VAUGHAN, J.H. (1972)
Procainamide induced lupus erythematosus - clinical and laboratory observations.
Am. J. Med. 52, 338.

BONANNI, P.P., FRYMOYER, J.W. & JACOX, R.F. (1965)
A family study of idiopathic pulmonary fibrosis. A possible dysproteinemic and genetically determined disease.
Amer. J. Med. 39, 411.

BONOMO, L., GILLARDI, U. & TURSI, A. (1966)
Anti gamma globulin and antinuclear factors in chronic bronchitis. Relation to disease course and hyperglobulinaemia.
Am. J. Clin. Pathol. 45, 313.

BONOMO, L., TURSI, A., TRIMIGLIOZZI, G. et al. (1965)
LE cells and antinuclear factors in leprosy.
Brit. Med. J. 2, 689.

BOREL, Y., LEWIS, R.M. & STOLLAR, B.D. (1973)
Prevention of murine lupus nephritis by carrier dependent induction of immunologic tolerance to denatured DNA.
Science 182, 76.

BRAUNSTEINER, H., EGGHART, F. & POLUZHEK, O. (1960)
Primär chronische Polyarthritis mit chronischen pulmonalen Veränderungen.
Dtsch. Med. Wochenschr. 85, 115.

BRAVERMAN, I.M. & MARINO, J. (1966)
Genetic aspects of autoimmune disease in NZB/NZW mice.
J. Clin. Invest. 45, 990.

BRITISH THORACIC & TUBERCULOSIS ASSOCIATION ASTHMA STUDY (1974).

BROGAN, T.D. (1972)
Leucocyte-migration test.
Lancet 1, 537.

BURRELL, R.G., WALLACE, J.P. & ANDREWS, C.E. (1964)
Lung antibodies in patients with pulmonary disease.
Am. Rev. Respir. Dis. 89, 697.

CAMMARATA, R.J., RODNAN, G.P. & FENNELL, R.H. (1967)
Serum anti-γ-globulin and ANF in the aged.
J. Am. Med. Assoc. 199, 455.

CANNAT, A. & SELIGMANN, M. (1965)
Les anticorps anti-nucléaires décelés par immunofluorescence et par d'autres méthodes immunologiques.
Sem. Hop. Paris 41, 1090.

CANNAT, A. & SELIGMANN, M. (1966)
Possible induction of antinuclear antibodies by isoniazid.
Lancet 1, 185.

CANNAT, A. & SELIGMANN, M. (1973)
Immunological abnormalities in juvenile myelomonocytic leukaemia.
Brit. Med. J. 1, 71.

CAPLAN, A. (1953)
Certain unusual radiological appearances in the chest of coal-miners suffering from RA.
Thorax 8, 29.

CAPLAN, A., PAYNE, R.B. & WITHEY, J.L. (1962)
A broader concept of Caplan's syndrome related to rheumatoid factors.
Thorax 17, 205.

CASALS, S.P., FRIOU, G.J. & TEAGUE, P.O. (1963)
Specific nuclear reaction pattern of antibody to DNA in lupus erythematosus sera.
J. Lab. Clin. Med. 62, 625.

CHACHAJ, W., LANGE, A., MALOLEPSZY, J. et al. (1973)
Antinuclear antibodies in serum of patients with bronchial asthma.
Pol. Tyg. Lek. 28, 1967.

CHANTLER, S., HANSEN, J., JACOBSON, J. et al. (1973)
Incidence of nuclear antibodies in patients and in related and unrelated groups from a community with a microepidemic of systemic lupus erythematosus.
Clin. Immunol. Immunopathol. 2, 9.

CHETSANGA, C.J., BOYD, V., PETERSON, L. et al. (1975)
Single-stranded regions in DNA of old mice.
Nature 253, 130.

CHRISTIAN, C.L., De SIMONE, A.R. & ABRUZZO, J.L. (1965)
Anti-DNA antibodies in hyperimmunized rabbits.
J. Exp. Med. 121, 309.

CLAUSEN, J. (1972)
"Immunochemical techniques for the identification and estimation of macromolecules."
Published by North-Holland Publishing Company, Amsterdam. p.526.

COCHRANE, C.G. & KOFFLER, D. (1973)
Immune complex disease in experimental animals and man.
Adv. Immunol. 16, 186.

COHEN, A.S. & CANOSO, J.J. (1972)
Criteria for the classification of systemic lupus erythematosus-status.
Arthritis Rheum. 15, 540.

COHEN, S.A., HUGHES, G.R.V., NOEL, G.L. et al. (1971)
Character of anti-DNA antibodies in systemic lupus erythematosus.
Clin. Exp. Immunol. 8, 551.

COOMBS, R.R.A. & GELL, P.G.H. (1975)
Classification of allergic reactions reponsible for clinical hypersensitivity and disease.
In "Clinical aspects of immunology".
Edited by Gell, P.G.H., Coombs, R.R.A. & Lachmann, P.J.
Published by Blackwell. 3rd edition. p.761-782.

COONS, A.H. & KAPLAN, M.H. (1950)
Localisation of antigens in tissue cells: improvements in methods for detection of antigen by means of fluorescent antibody.
J. Exp. Med. 91, 1.

CORMANE, R.H. (1964)
"Bound" globulin in the skin of patients with chronic discoid lupus erythematosus and systemic lupus erythematosus.
Lancet 1, 534.

COULSON, A.S. & CHALMERS, D.G. (1964)
Separation of viable lymphocytes from human blood.
Lancet 1, 468.

CURTAIN, C.C. (1958)
Electrophoresis of fluorescent antibody.
Nature 182, 1305.

DACIE, J.V. & LEWIS, S.M. (1963)
"Practical Haematology". Published by Churchill, London. 3rd edition. p.3.

DACIE,J.V. & LEWIS, S.M. (1970)
"Practical Haematology". Published by Churchill Livingstone. 4th edition. p.245.

DACIE, J.V. & LEWIS, S.M. (1970)
"Practical Haematology". Published by Churchill Livingstone. 4th edition. p.246.

DAVIS,B.D., DULBECCO, R., EISEN, H.N. et al. (1968)
Lysogeny, episomes and transducing bacteriophages.
In "Principles of Microbiology and Immunology".
Published by Harper International.
Chapter 24. p.728.

DAVIS, J.M. (1967)
The effects of crysotile asbestos dust on lung macrophages maintained in organ culture.
Br. J. Exp. Pathol. 48, 379.

DAVIS, J.S. (1971)
Determination of DNA and anti-DNA by counterimmuno-electrophoresis.
Arthritis Rheum. 14, 377.

DAVIS, J.S. (1975)
Measurement of anti-DNA by complement fixation, precipitation, passive hemagglutination, counter immunoelectrophoresis and immunosorbant assays.
Scand. J. Rheumatol. Suppl. 11, 20.

De HORATIUS R.J., ABRUZZO, J.L. & WILLIAMS, R.C. (1972)
Immunofluorescent and immunologic studies of rheumatoid lung.
Arch. Intern. Med. 129, 441.

DETTLAF, Z., BOWSZYE, J. & WIECKI, J. (1973).
Antinuclear antibodies in the sera of in patients of the pulmonary tuberculosis clinic.
Gruzlica 41, 15.

DINU, T.V. & ROTH, L. (1965)
Contribution to the auto-immune pathogeny of silicosis.
Med. Interna (Bucur.) 17, 1207.

DIXON, F.J., OLDSTONE, M.B.A. & TONIETTI, G. (1971)
Pathogenesis of immune complex nephritis of New Zealand mice.
J. Exp. Med. 134: Suppl: 65S-71S.

DONIACH, D., ROITT, I.M., WALKER, J.G. et al. (1966)
Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications.
Clin. Exp. Immunol. 1, 237.

DRESSER, D.W., & MITCHISON, N.A. (1968)
The mechanism of immunological paralysis.
Ad. Immunol. 8, 129.

EAGLSTEIN, W.H.; REASOR, H.S. & HSIA, S.L. (1969)
Membrane stabilization: effects of hydrocortisone sodium succinate on phospholipase C-treated sheep erythrocytes.
Proc. Soc. Exp. Biol. Med. 132, 919.

ELLMAN, P., & BALL, R.E. (1948)
"Rheumatoid disease" with joint and pulmonary manifestations.
Br. Med. J. 2, 816.

ERNSTROM, U. & LARSSON, B. (1967)
Export and import of lymphocytes in the thymus during steroid-induced involution and regeneration.
Acta Pathol. Microbiol. Scand. /B/ 70, 371.

FABER, V., ELLING, P., NORUP, G. et al. (1964)
An antinuclear factor specific for leucocytes.
Lancet 2, 344.

FALK, R.E., COLLSTE, L. & MÖLLER, G. (1970)
In vitro detection of transplantation immunity: the inhibition of migration of immune spleen cells and peripheral blood leucocytes by specific antigen.
J. Immunol. 104, 1287.

FALON, J.T. (1937)
Specific tissue reaction to phospholipids: suggested explanation for similarity of lesions of silicosis and pulmonary tuberculosis.
Can. Med. Assoc. J. 36, 223.

FARR, R.S. (1958)
A quantitative immunochemical measure of the primary interaction between I* BSA and antibody.
J. Infect. Dis. 103, 239.

FEDERLIN, K., MAINI, R.N., RUSSELL, D.S. et al. (1971)
A micromethod for peripheral leucocyte migration in tuberculin sensitivity.
J. Clin. Pathol. 24, 533.

FELTKAMP, T.E.W. (1966)
Idiopathic autoimmune diseases - a study of their serological relationship.
Academisch Proefscrift. Drukkerij. Aemstelstad. Amsterdam.

FORBES, I.J. (1958)
Diffuse interstitial pulmonary fibrosis and honeycomb lung.
Australas. Ann. Med. 7, 205.

FOULIS, A.K. (1972)
Leucocyte-migration test.
Lancet 1, 842.

FRANKLIN, E.C., ZUCKE-FRANKLIN D. & McEWEN C. (1959)
Some observations on patients with interstitial pulmonary fibrosis.
Arch. Interam. Rheum. (Rio de J.) 2, 250.

FREEDMAN, P. & MARKOWITZ, A.S. (1959)
Immunological studies in nephritis.
Lancet 2, 45.

FREEDMAN, P. & MARKOWITZ, A.S. (1962)
Isolation of antibody-like γ globulin from lupus glomeruli.
Br. Med. J. 1, 1175.

FRIOU, G.J. (1958)
Clinical application of a test for lupus globulin-nucleohistone interaction using fluorescent antibody.
Yale J. Biol. Med. 31, 40.

FRIOU, G.J. (1958)
The significance of the lupus globulin - nucleoprotein reaction.
Ann. Intern. Med. 49, 866.

FRIOU, G.J. (1967)
Antinuclear antibodies: diagnostic significance and methods.
Arthritis Rheum. 10, 151.

FRIOU, G.J. & TEAGUE, P.O. (1963)
Spontaneous autoimmunity in mice: antibodies to nucleoprotein in strain A-J.
Science 143, 1333.

FROST, P.G. & LACHMANN, P.J. (1968)
The relationship of desoxyribonuclease inhibitor levels in human sera to the occurrence of antinuclear antibodies.
Clin. Exp. Immunol. 3, 447.

GALANAUD, P., DORMONT, J., CROSNIER, J. et al. (1971)
Leucocyte-migration test in seronegative S.L.E.
Lancet 2, 923.

GAMBINI, G. (1959)
In "Atti del XXII Congresso Nazionall di medicina Lavora".
Editors E.C. Vigliani et al. p.107

GEISERT, M., HEICKE, B., METZMANN, E. et al. (1975)
Influence of molecular weight of DNA on the determination of anti-DNA antibodies in systemic lupus erythematosus (SLE) sera by radioimmunoassay.
Nucleic Acids Research 2, 521.

GEORGE, M. & VAUGHAN, J.H. (1962)
In vitro cell migration as a model for delayed hypersensitivity.
Proc. Soc. Exp. Biol. Med. 111, 514.

GILLIAM, J.N., CHEATUM, D.E., HURD, E.R. et al. (1974)
Immunoglobulin in clinically uninvolved skin in systemic lupus erythematosus. Association with renal disease.
J. Clin. Invest. 53, 1434.

GLASS, D.N., CAFFIN, J., MAINI, R.N. et al. (1973)
Measurement of DNA antibodies by double antibody precipitation.
Ann. Rheum. Dis. 32, 342.

GLYNN, L.E. (1975)
Rheumatoid arthritis.
In "Clinical aspects of immunology".
Edited by Gell, P.G.H. & Coombs, R.R.A.
Published by Blackwell. 3rd edition. p.1099-1116.

GODEAU, P., AUBERT, M., IMBERT, J.C. et al. (1973)
Systemic lupus erythematosus and active izoniazid levels. Report of 47 cases.
Ann. Intern. Med. 124, 181.

GOLDEN, A. & TULLIS, I.F.Jr. (1949)
Diffuse interstitial fibrosis of the lungs.
Mil. Surgeon 105, 130.

GONZALEZ, E.N., & ROTHFIELD, N.F. (1966)
Immunoglobulin class and pattern of nuclear fluorescence in systemic lupus erythematosus.
N. Engl. J. Med. 274, 133.

GOUGH, J., RIVERS, D. & SEAL, R.M.E. (1955)
Pathological studies of modified pneumoconiosis in coal-miners with rheumatoid arthritis. (Caplan's syndrome).
Thorax 10, 9.

GROSSMAN, J., CALLERAME, M.L. & CONDEMI, J.J. (1974)
Skin immunofluorescence studies on lupus erythematosus and other antinuclear antibody positive diseases.
Ann. Intern. Med. 80, 496.

HABEEB, W.J. (1945)
Eosinophilia in silicosis.
Am. Rev. Tuberc. 52, 337.

HADJIYANNAKI, K. & LACHMANN, P.J. (1972)
The relation of deoxyribonuclease inhibitor levels to the occurrence of antinuclear antibodies in NZB/NZW mice.
Clin. Exp. Immunol. 11, 291.

HAGADORN, J.E. & BURRELL, R. (1968)
Lung-reactive antibodies in IgA fractions of sera from patients with pneumoconiosis.
Clin. Exp. Immunol. 3, 263.

HAHN, B.H., BAGBY, M.K. & OSTERLAND, C.K. (1973)
Abnormalities of delayed hypersensitivity in systemic lupus erythematosus.
Am. J. Med. 55, 25.

HALL, R., TURNER-WARWICK, M. & DONIACH, D. (1966)
Autoantibodies in iodide goitre and asthma.
Clin. Exp. Immunol. 1, 285.

HARGRAVES, M.M., RICHMOND, H. & MORTON, R. (1948)
Presentation of two bone marrow elements: the "tart" cell and the "LE" cell.
Mayo Clin. Proc. 23, 25.

HARPEY, J.P., CAILLE, B., MOULIAS, R. et al. (1971)
Lupus-like syndrome induced by d-penicillamine in Wilson's disease.
Lancet 1, 292.

HARVEY, A.M., SHULMAN, L.E., TUMULTY, P.A. et al. (1954)
Systemic lupus erythematosus; review of the literature and clinical analysis of 138 cases.
Medicine (Baltimore) 33, 291.

HASLAM, P.L., GRIFFITHS, I.D., TURNER-WARWICK, M. et al. (1976)
Antibodies to deoxyribonucleic acid in systemic lupus erythematosus.
In preparation.

HASLAM, P.L., SOUTAR, C. & TURNER-WARWICK, M. (1976)
IgE ANA in lung disease.
(Unpublished).

HEARD, B.E. (1962)
Fibrous healing of old iatrogenic pulmonary oedema ('hexamethonium lung').
J. Pathol. Bacterial. 83, 159.

HENCH, P.K., TAN, E.M. & WILSON C.B. (1969)
In vivo fixation of gamma globulin in renal cell nuclei of patients with systemic lupus orythematosus (SLE).
Arthritis Rheum. /Abstracts/ 12, 668.

HEPPLESTRON, A.G. (1969)
The fibrogenic action of silica.
Br. Med. Bull. 25, 282.

HEPPLESTRON, A.G. & STYLES, J.A. (1967)
Activity of a macrophage factor in collagen formation by silica.
Nature 214, 521.

HOBBS, J.R. & TURNER-WARWICK, M. (1967)
Assay of circulating immunoglobulins in patients with fibrosing alveolitis.
Clin. Exp. Immunol. 2, 645.

HODSON, M.E. & TURNER-WARWICK, M. (1975)
Autoantibodies in patients with bronchial carcinoma.
Thorax 30, 367.

HODSON, M.E. & TURNER-WARWICK, M. (1976)
Autoantibodies in patients with chronic bronchitis.
Br. J. Dis. Chest 70, in press.

HOFFBRAND, B.I. (1965)
"Unexplained" dyspnoea and shrinking of the lungs in SLE.
Br. Med. J. 1, 1273.

HOLBOROW, E.J. & JOHNSON, G.D. (1965)
The nature of antinuclear immunoglobulins.
Ann. NY Acad. Sci. 124, 833.

HOLBOROW, E.J. & JOHNSON, G.D. (1967)
Immunofluorescence.
In "Handbook of Experimental Immunology".
Edited by Weir, D.M.
Published by Blackwell Scientific Publications. p.587.

HOLGATE, S., GLASS, D., HASLAM, P. et al. (1976)
Respiratory involvement in systemic lupus erythematosus. A clinical and immunological study.
Clin. Exp. Immunol. (In press).

HOLIAN, J., GRIFFITHS, I.D., GLASS, D.N. et al. (1975)
Human anti-DNA antibody reference standards for diagnosis and management of systemic lupus erythematosus
Ann. Rheum. Dis. 34, 438.

HOLMAN,H. & Deicher, H.R. (1959)
The reaction of lupus erythematosus (LE) cell factor with deoxyribonucleoprotein of the cell nucleus.
J. Clin. Invest. 38, 2059.

HOOPER, B., WHITTINGHAM, S., MATHEWS, J.D. et al. (1972)
Autoimmunity in a rural community.
Clin. Exp. Immunol. 12, 79.

HORWITZ, D.A. (1972)
Impaired delayed hypersensitivity in systemic lupus erythematosus.
Arthritis Rheum. 15, 353.

HOWARD, J.G. & MITCHISON, N.A. (1975)
Immunological tolerance.
Prog. Allergy 18, 43.

HOWIE, J.B. (1973)
Autoimmune disease in mice.
In "Textbook of Immunopathology".
Edited by Meischer, P.A. and Müller-Eberhard, H.J.
Published by Grune and Stratton, New York and London.
Volume 1 p.179-188.

HUANG, C.T. & LYONS, H.A. (1966)
Comparison of pulmonary function in patients with systemic lupus erythematosus, scleroderma and rheumatoid arthritis.
Am. Rev. Respir. Dis. 93, 865.

HUGHES, G.R.V. (1971)
Significance of anti-DNA antibodies in SLE.
Lancet 2, 861.

HUGHES, G.R.V. (1975)
Frequency of anti-DNA antibodies in SLE, RA and other diseases.
Scand. J. Rheumatol. Suppl. 11, 42.

HUGHES, G.R.V., COHEN, S.A. & CHRISTIAN, C.L. (1971)
Anti-DNA activity in systemic lupus erythematosus.
Ann. Rheum. Dis. 30, 259.

HUGHES, G.R.V., COHEN, S.A., LIGHTFOOT, R.W. et al. (1971)
The release of DNA into serum and synovial fluid.
Arthritis Rheum. 14, 259.

HUGHES, P. & ROWELL, N.R. (1970)
Aggravation of turpentine-induced pleurisy in rats by "homogeneous" and "speckled" antinuclear antibodies.
J. Pathol. 101, 141.

HYUN, B.H., DIGGS, C.L. & TOONE, E.C. (1962)
Dermatomyositis with cystic fibrosis (honeycombing) of the lung. Case report.
Dis. Chest 42, 449.

ISRAEL, H.L. (1953)
Pulmonary manifestations of disseminated lupus erythematosus.
Am. J. Med. Sci. 226, 387.

JAYSON, M.I., DONIACH, D., BENHAMOU-GLYNN, N. et al. (1967)
Thryotoxicosis and Hashimoto goitre in a pair of monozygotic twins with serum long-acting thyroid stimulator.
Lancet 2, 15.

JOHNS, E.W., PHILLIPS, D.M.P., SIMSON, P. et al. (1960)
Improved fractions of arginine-rich histones from calf thymus.
Biochem. J. 77, 631.

JOHNSON, G.D., EDMONDS, J.P. & HOLBOROW, E.J. (1973)
Precipitating antibody to DNA detected by two-stage electro immuno-diffusion. Study in SLE and in rheumatoid arthritis.
Lancet 2, 883.

JOHNSON, G.D. & HOLBOROW, E.J.
Immunofluorescence.
In "Handbook of experimental immunology"
Volume 1. Immunochemistry.
Edited by Weir, D.M.
Published by Blackwell Scientific publications.
Second edition. Chapter 18.

JOKINEN, E.J. & JULKUNEN, H. (1965)
DNA haemagglutination test in the diagnosis of systemic lupus erythematosus.
Ann. Rheum. Dis. 24, 477.

JONES, R.N., TURNER-WARWICK, M., WEILL, H. et al. (1976)
Autoimmune disorders in accelerated silicosis.
Am. J. Med. /Abstracts/. In press.

KALMAN, E., MANDI, A. & OSZTOVICS, M. (1962)
The investigation of antilung antibodies in silicosis.
Med. Lav. 53, 165.

KAMIJIMA, K. (1968)
Silicosis as an adjuvant disease of man.
Acta Med. Shikoku 24, 513.

KANG, K.Y., YAGURA, T., SERA, Y. et al. (1973)
Antinuclear factor in pneumoconioses and idiopathic pulmonary fibrosis.
Med. J. Osaka Univ. 23, 249.

KANTOR, G.L., Van HERLE, A.J. & BARNETT, E.V. (1970)
Autoantibodies of the IgD class.
Clin. Exp. Immunol. 6, 951.

KARLISH, A.J. (1969)
Lung changes in Sjögrens syndrome.
Proc. R. Soc. Med. 62, 1042.

KLIPPEL, J.H., GRIMLEY, P.M., DECKER, J.L. et al. (1974)
Lymphocyte tubuloreticular structures in lupus erythematosus. Correlation with disease activity.
Ann. Intern. Med. 81, 355.

KNIKER, W.T. & COCHRANE, C.G. (1968)
The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces.
J. Exp. Med. 127, 119.

KOFFLER, D. (1974)
Immunopathogenesis of systemic lupus erythematosus.
Ann. Rev. Med. 25, 149.

KOFFLER, D., AGNELLO, V. & KUNKEL, H.G. (1974)
Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus.
Am. J. Pathol. 74, 109.

KOFFLER, D., AGNELLO, V., WINCHESTER, R. et al. (1973)
The occurence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases.
J. Clin. Invest. 52, 198.

KOFFLER, D., CARR, R.I., AGNELLO, V. et al. (1969)
Antibodies to polynucleotides: distribution in human serums.
Science 166, 1648.

KOFFLER, D., CARR, R., AGNELLO, V. et al. (1971)
Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease.
J. Exp. Med. 134, 294.

KOFFLER, D., SCHUR, P.H. & KUNKEL, H.G. (1967)
Immunological studies concerning the nephritis of systemic lupus erythematosus.
J. Exp. Med. 126, 607.

KRISHMAN, C. & KAPLAN, M.H. (1966)
Anti-nuclear activity in acid eluates of glomeruli from lupus nephritis kidneys.
Fed. Proc. 25, 309.

KRISHMAN, C. & KAPLAN, M.H. (1967)
Immunopathologic studies of systemic lupus erythematosus.
11. Anti-nuclear reaction of γ globulin eluted from homogenates and isolated glomeruli of kidneys from patients with lupus nephritis.
J. Clin. Invest. 46, 569.

LACHMANN, P.J., MÜLLER-EBERHARD, H.J., KUNKEL, H.G. et al. (1962)
The localization of in vivo bound complement in tissue sections.
J. Exp. Med. 115, 63.

LACOUR, F., MAHON- MERLIN, E. & MICHELSON, M. (1973)
Immunological recognition of polynucleotide structure.
In "Current Topics in Microbiology and Immunology".
Published by Springer-Verlag, New York.
Volume 62, p.1-39.

LANGE, A., SMOLIK, R., ZATONSKI, W. et al. (1974)
Autoantibodies and serum immunoglobulin levels in asbestos workers.
Int. Arch. Arbeitsmed. 32, 313.

LANZINGER, G., RITZ, E., FRANZ, H.E. et al. (1969)
Acute interstitial pulmonary fibrosis in a patient with Paraquat poisoning. Clinico anatomical observation of a case of Paraquat poisoning which had a fatal outcome.
Munch. Med. Wochenschr. 111, 944.

LIGHTFOOT, R.W., REDECHA, P.B. & LEVESANOS, N. (1975)
Longitudinal studies of anti-DNA antibody levels in SLE.
Scand. J. Rheumatol. Suppl. 11, 52.

LINDQUIST, K.J., COLEMAN, R.E. & OSTERLAND, C.K. (1970)
Autoantibodies in chronic pulmonary tuberculosis.
J. Chronic Dis. 22, 717.

LING, N.R. (1968)
"Lymphocyte stimulation".
Published by North Holland, Amsterdam.

LIPPMANN, M., ECKERT, H.L., HAHON, N. et al. (1973)
Circulating antinuclear and rheumatoid factors in coal miners. A prevalence study in Pennsylvania and West Virginia.
Ann. Intern. Med. 79, 807.

LITTLER, W.A. & OGILVIE, C. (1970)
Lung function in patients receiving busulphan.
Br. Med. J. 4, 530.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. et al. (1951)
Protein measurement with folin phenol reagent.
J. Biol. Chem. 193, 265.

LUCIANO, A. & ROTHFIELD, N.F. (1973)
Patterns of nuclear fluorescence and DNA-binding activity.
Ann. Rheum. Dis. 32, 337.

MACKAY, I.R. & BURNET, McF. (1963)
Autoimmune aspects of some chronic "inflammatory" and chronic systemic diseases: Pulmonary fibrosis.
In "Autoimmune Diseases".
Published by Charles C. Thomas.
P. 226.

MACKAY, I.R. & RITCHIE, B. (1965)
Diffuse fibrosing alveolitis (diffuse interstitial fibrosis of the lungs) : two cases with autoimmune features.
Thorax 20, 200.

MACLENNAN, I.C. (1972)
Antibody in the Induction and Inhibition of Lymphocyte Cytotoxicity.
Transplantation Rev. 13, 67.

MAINI, R.N., ROWE, L.M., MAGRATH, I.T. et al. (1973)
Standardization of the leucocyte migration test.
Int. Arch. Allergy 45, 308.

MAKELA, O. & NOSSAL, G.J. (1961)
Bacterial adherence: a method for detecting antibody production by single cells.
J. Immunol. 87, 447.

MARMUR, J. (1961)
A procedure for the isolation of deoxyribonucleic acid from micro-organisms.
J. Mol. Biol. 3, 208.

MARSHALL, J.D., EVELAND, W.C. & SMITH, C.W. (1958)
Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technic with a modification of its application.
Proc. Soc. Exp. Biol. Med. 98, 898.

MATHEWS, J.D., HOOPER, B.M., WHITTINGHAM, S. et al. (1973)
Association of autoantibodies with smoking, cardiovascular morbidity, and death in the Busselton population.
Lancet 2, 754.

MATTIOLI, M. & REICHLIN, M. (1974)
Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus.
Arthritis Rheum. 17, 421.

MEDICAL RESEARCH COUNCIL COLLAGEN DISEASES & HYPERSENSITIVITY PANEL (1961)
Treatment of systemic lupus erythematosus with steroids.
Br. Med. J. 2, 915.

MEDICAL RESEARCH COUNCIL REPORT (1965)
Definition and classification of chronic bronchitis for clinical and epidemiological purposes.
Lancet 1, 775.

MERCHANT, J.A., KLOUDA, P.T., SOUTAR, C.A. et al. (1975)
The HL-A system in asbestos workers.
Br. Med. J. 1, 189.

METZ, G. & METZ, J. (1974)
Intracytoplasmic ultrastructures in peripheral blood cells in lupus erythematosus.
Arch. Dermatol. Forsch. 249, 35.

MIYAWAKI, S. & RITCHIE, R.F. (1974)
Heterogeneity of antinucleolar antibody and IgE antinuclear antibody in patients with systemic rheumatic diseases.
J. Immunol. 113, 1346.

MÖLLER, G. (1969)
Induction of DNA synthesis in normal human lymphocyte cultures by antigen-antibody complexes.
Clin. Exp. Immunol. 4, 65.

MOOLTEN, S.E. (1974)
Autoimmune phenomena in evolution of systemic lupus erythematosus reflections on its interpretation as a slow virus infection.
Mt. Sinai J. Med. N.Y. 41, 505.

MOULIAS, R., GOUST, J.M., DEVILLE-CHABROLLE, A. et al. (1970)
Le test de migration des leucocytes du sang périphérique (T.M.L.). Un nouveau test d'hypersensibilité retardée in vitro chez l'homme. II. Utilization dans les affectations auto-immunes humaines. Résultats préliminaires et interprétation.
Pressé Méd. 78, 2315.

MULLER-BERAT, C.N. & MOULIAS, R. (1970)
Le test de migration des leucocytes du sang périphériques (T.M.L.). Un nouveau test d'hypersensibilite retardée in vitro chez d'applications pratique.
Pressé Méd. 78, 77.

NAGAYA, H., BUCKLEY, C.F. & SIEKER, H.O. (1969)
Positive antinuclear factor in patients with unexplained pu monary fibrosis.
Ann. Intern. Med. 70, 1135.

NAHON, M.E., MOULART, L. & LACOUR, F. (1973)
Immunochemical study of anti poly I: poly C antibodies and of their reaction with RNA.
Immunochemistry 10, 571.

NAKAMURA, R.M. & ALLEN Jr. H.J. (1973)
Immunopathologic mechanisms and recent concepts in the pathogenesis of systemic lupus erythematosus.
Cutis (N.Y.) 11, 607.

NASSONOVA, V.A., ALEKBEROVA, Z.S., POVERENNY, A.M. et al. (1972)
The clinical significance of determining antibodies (poly A: poly U) in systemic lupus erythematosus.
Ter. Arkh. 44, 24.

NELSON, D.S. (1974)
Antigens of carcinoma of the cervix uteri. A study by means of immunofluorescence.
Clin. Exp. Immunol. 16, 53.

NICKLAUS, T.M. & SNYDER, A.B. (1968)
Nitrofurantoin pulmonary reaction. A unique syndrome.
Arch. Intern. Med. 121, 151.

NOZZOLI, F. & LAMANNA, A. (1966)
Antiphosphatide antibodies in acute and chronic pulmonary diseases and in non tuberculous pleuropneumopathies (a preliminary study).
Settimana Med. 54, 989.

OLSEN, E.G.J. & LEVER, J.V. (1970)
Systemic lupus erythematosus: pulmonary histology.
Thorax 25, 509.

PARKER, L.P., HAHN, B.H. & OSTERLAND, C.K. (1974)
Modification of NZB/NZW F_1 autoimmune disease by development of tolerance to DNA.
J. Immunol. 113, 292.

PARKER, M.D. (1973)
Ribonucleoprotein antibodies: frequency and clinical significance in systemic lupus erythematosus, scleroderma and mixed connective tissue disease.
J. Lab. Clin. Med. 82, 769.

PARONETTO, F. & KOFFLER, D. (1965)
Immunofluorescent localization of immunoglobulins, complement and fibrinogen in human diseases.
1. Systemic lupus erythematosus.
J. Clin. Invest. 44, 1657.

PATRUCCO, A., ROTHFIELD, N.F. & HIRSCHORN, K. (1967)
The response of cultured lymphocytes from patients with SLE to DNA.
Arthritis Rheum. 10, 32.

PEABODY, J.W., PEABODY, J.W. Jr., HAYES, E.W. et al. (1950)
Idiopathic pulmonary fibrosis: its occurrence in twin sisters.
Dis. Chest 18, 330.

PELTIER, A.P., HUGHES, G.R.V., KAHN, M.F. et al. (1974)
Detection of serum antidesoxyribonucleic acid antibodies by Farr's radio immuno assay.
Nouv. Presse Med. 3, 649.

PEPYS, J. (1967)
Hypersensitivity to inhaled organic antigens.
J. R. Coll. Physicians Lond. 2, 42.

PEPYS, J. (1969)
Hypersensitivity disease of the lungs due to fungi and organic dusts.
In "Monographs in Allergy".
Edited by S. Karger.
Volume 4.

PERNIS, B. (1963)
Immunohistochemical observations on the human silicotic nodule.
Med. Lav. 54, 354.

PERNIS, B., CHIAPPINO, G., GILSON, G.C. et al. (1965)
Studies on Caplan's nodules by means of immunofluorescence.
Grandfr. Silikoseforsch. 6, 339.

PERNIS, B. & PARONETTO, F. (1962)
Adjuvant effect of silica (tridymite) on antibody production.
Proc. Soc. Exp. Biol. Med. 110, 390.

PERNIS, B., VIGLIANI, E.C. and GAMBINI, G. (1963)
Rheumatoid factors and pneumoconiosis.
Grundfr. Silikoseforsch. 5, 471.

PERNIS,B., VIGLIANI, E.C., MARCHISIO, M.A. et al. (1966)
Observations on the effect of asbestos on cells in vitro.
Med. Lav. 57, 721.

PERNIS, B., VIGLIANI, E.C. & SELIKOFF, I.J. (1965)
Rheumatoid factor in the serum of individuals exposed to asbestos.
Ann. N.Y. Acad. Sci. 132, 112.

PINCUS, T., SCHUR, P.H., ROSE, J.A. et al. (1969)
Measurement of serum DNA-binding activity in systemic lupus erythematosus.
N. Engl. J. Med. 281, 701.

POKORNEY, C. & HELLWIG, C.A. (1955)
Diffuse interstitial fibrosis of the lungs.
Arch. Pathol. 59, 382.

POLLAK, V.E., MANDEMA, E. & KARK, R.M. (1960)
Antinuclear factors in the serum of relatives of patients with systemic lupus erythematosus.
Lancet 2, 1061.

PONDMAN, K.W., ES, L. van. & SCHUURS, A. (1965)
The relationship between complement, the heterogeneity of antibody and anaphylaxis.
Bibl. Haematol. 23, 825.

POWELL, D.E. & STEINBERG, A.D. (1972)
The pathogenesis of autoimmunity in New Zealand mice. IV. Independent stimulation of antibodies to DNA and RNA.
Clin. Exp. Immunol. 12, 419.

PURITZ, E.M., YOUNT, W.J., NEWELL, M. et al. (1973)
Immunoglobulin classes and IgG subclasses of human antinuclear antibodies. A correlation of complement fixation and the nephritis of systemic lupus erythematosus.
Clin. Immunol. Immunopathol. 2, 98.

QUISMORIO, F.P., BLAND, S.L. & FRIOU, G.J. (1971)
Autoimmunity in thermal injury: occurrence of rheumatoid factors, antinuclear antibodies and anti-ephithelial antibodies.
Clin. Exp. Immunol. 8, 701.

RAM, J.S. (1967)
Aging and immunological phenomena - a review.
J. Gerontol. 22, 92.

READ, J. (1958)
The pathogenesis of the Hamman-Rich Syndrome. A review from the standpoint of possible allergic etiology.
Am. Rev. Tuberc. 78, 353.

REICHLIN, M. & MATTIOLI, M. (1972)
Correlation of a precipitin reaction to an RNA protein antigen and a low prevalence of nephritis in patients with systemic lupus erythematosus.
N. Engl. J. Med. 286, 908.

REICHLIN, M. & MATTIOLI, M. (1974)
Antigens and antibodies characteristic of systemic lupus erythematosus.
Bull. Rheum. Dis. 24, 756.

REINERT, P., DEVILLECHABROLLE, A., BRIBET-FORETTE, F. et al. (1971)
Le test de transformation blastique des lymphocytes (TTL) appliqué au diagnostic des affectations autoimmunes. Premiers résultats en présence d'antigènes thyroidiens et d'ADN.
Presse Med. 79, 1279.

RITCHIE, R.F. (1967)
The clinical significance of titered antinuclear antibodies.
Arthritis Rheum. 10, 544.

ROBBINS,W.C., HOLMAN, H.R., DEICHER, H.R. et al. (1957)
Complement fixation with cell nuclei and DNA in lupus erythematosus.
Proc. Soc. Exp. Biol. Med. 96, 575.

ROBITAILLE, P. (1974)
Specific radioimmunoassay allowing simultaneous detection of antibodies to soluble nucleoprotein (NPS) and native desoxyribonucleic acid (ADN N).
Union Med. Can. 103, 691.

ROBITAILLE, P., ZVAIFLER, N.J. & TAN, E.M. (1973)
Antinuclear antibodies and nuclear antigens in rheumatoid synovial fluids.
Clin. Immunol. Immunopathol. 1, 385.

ROTHFIELD, N.F. & RODNAN, G.P. (1968)
Serum antinuclear antibodies in progressive systemic sclerosis (scleroderma).
Arthritis Rheum. 11, 607.

ROTHFIELD, N.F. & STOLLAR, B.D. (1967)
The relation of immunoglobulin class, pattern of antinuclear antibody and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus.
J. Clin. Invest. 46, 1785.

ROY, L.P., WESTBERG, N.G. & MICHAEL, A.F. (1973)
Nephrotic syndrome: No evidence of a role for IgE.
Clin. Exp. Immunol. 13, 553.

SANDBERG, A.L. & STOLLAR, B.D. (1966)
Comparisons of antibodies reacting with DNA.
II. Rabbit antibodies induced by nucleoside-protein conjugates.
J. Immunol. 96, 764.

SCADDING, J.G. (1964)
Fibrosing alveolitis.
Br. Med. J. 2, 686.

SCADDING, J.G. (1969)
The lungs in rheumatoid arthritis.
Proc. R. Soc. Med. 62, 227.

SCHEIDEGGER, J.J. (1955)
Une micro-methode de l'immuno-electrophorese.
Int. Arch. Allergy 7, 103.

SCHUR, P.H., MOROZ, L.A. & KUNKEL, H.G. (1968)
Precipitating antibodies to ribosomes in serum of patients with systemic lupus erythematosus.
Immunochemistry 4, 447.

SCHUR, P.H. & SANDSON, J. (1968)
Immunologic factors and clinical activity in systemic lupus erythematosus.
N. Engl. J. Med. 278, 533.

SCHWARTZ, R.S. (1975)
Viruses and systemic lupus erythematosus
N. Engl. J. Med. 293, 132.

SELIGMANN, M. & MILGROM, F. (1957)
Mise en évidence par la fixation du complément de la réaction entre acide desoxyribonu léique et le sérum de malades atteints de lupus érythemateux disséminé.
C. R. Soc. Biol. (Paris) 245, 1472.

SERAFINI, U., TORRIGIANI, G. & MASALA, C. (1964)
"The incidence of autoantibodies in the normal population".
In "Proceedings of the Vth International Congress of Allergology, Madrid, October, 1964".

SERAFINI, U., TORRIGIANI, G. & MASALA, C. (1965)
Organ-specific autoantibodies in reagin-containing sera from allergic patients.
Lancet 2, 321.

SHARP, G.C., IRWIN, W.S., TAN, E.M. et al. (1972)
Mixed connective tissue disease. An apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA).
Am. J. Med. 52, 148.

SØBORG, M. & BENDIXEN, G. (1967)
Human lymphocyte migration as a parameter of hypersensitivity.
Acta. Med. Scand. 181, 247.

SOUTAR, C.A., SIMON, G. & TURNER-WARWICK, M. (1974)
The radiology of asbestos-induced disease of the lungs.
Br. J. Dis. Chest 68, 235.

SOUTAR, C.A., TURNER-WARWICK, M. & PARKES, W.R. (1974)
Circulating antinuclear antibody and rheumatoid factor in coal pneumoconiosis.
Br. Med. J. 3, 145.

SPINAZZOLA, A., ZEDDA, S., LARIA, U. et al. (1968)
Association of silicosis and rheumatoid arthritis (with special reference to Sardinian miners).
Folia Med. Napoli 51, 984.

STACK, B.H.R., CHOO-KANG, Y.F.J. & HEARD, B.E. (1972)
The prognosis of cryptogenic fibrosing alveolitis.
Thorax 27, 535.

STIRPE, F. & ALDRIDGE, W.N. (1961)
Diphosphopyridine nucleotide pyrophosphorylase in the nuclei isolated from poisoned and regenerating rat liver.
Biochem. J. 80, 481.

STOLLAR, D. & LEVINE, L. (1961)
Antibodies to denatured deoxyribonucleic acid in lupus erythematosus serum.
J. Immonol. 87, 477.

STOLLAR, B.D. & SANDBERG, A.L. (1966)
Comparisons of antibodies reacting with DNA.
1. Systemic lupus erythematosus sera and rabbit antibodies induced by DNA-methylated bovine serum albumin complexes.
J. Immunol. 96, 755.

STRANDER, H. & CANTELL, K. (1966)
Production of interferon by human leucocytes in vitro.
Ann. Med. Exp. Biol. Fenn. 44, 265.

SWAYE, P., VAN ORDSTRAND, H.S., McCORMACK, L.J. et al. (1969)
Familiar Hamman-Rich Syndrome. Report of eight cases.
Dis. Chest 55, 7.

SYLVESTER, R.A., ATTIAS, M., TALAL, N. et al. (1973)
Antibodies to viral and synthetic double stranded RNA in discoid lupoid erythematosus.
Arthritis Rheum. 16, 383.

TALAL, N. (1973)
Antibodies binding ^{3}H reovirus RNA in systemic lupus erythematosus.
Clin. Immunol. Immunopathol. 1, 230.

TALAL, N. & STEINBERG, A.D. (1974)
The pathogenesis of autoimmunity in New Zealand black mice.
Curr. Top. Microbiol. Immunol. 64, 79.

TAN, E.M. (1968a)
The influence of hydralazine on nuclear antigen-antibody reactions.
Arthritis Rheum. 11, 515.

TAN, E.M. (1968b)
Antibodies to deoxyribonucleic acid irradiated with ultraviolet light: detection by precipitins and immunofluorescence.
Science 161, 1353.

TAN, E.M. (1973)
The clinical significance of antinuclear antibodies.
Postgrad. Med. 54, 143

TAN, E.M. & KUNKEL, H.G. (1966)
Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus.
J. Immunol. 96, 464.

TAN, E.M. & KUNKEL, H.G. (1966)
An immunofluorescent study of the skin lesions in systemic lupus erythematosus.
Arthritis Rheum. 9, 37.

TAN,E.M. & NATALI, P.G. (1970)
Comparative study of antibodies to native and denatured DNA.
J. Immunol. 104, 902.

TAN, E.M., SCHUR,P.H., CARR, R.T. et al. (1966)
Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus.
J. Clin. Invest. 45, 1732.

TOJO, T. & FRIOU, G.J. (1968)
Lupus nephritis: varying complement fixing properties of immunoglobulin G antibodies to antigens of cell nuclei.
Science 161, 904.

TURNER-WARWICK, M. (1967)
Auto-allergy and lung diseases.
J. R. Coll. Physicians Lond. 2, 57.

TURNER-WARWICK, M. (1968)
Fibrosing alveolitis and chronic liver disease.
Q. J. Med. 37, 133.

TURNER-WARWICK, M. (1969)
Rheumatoid arthritis rheumatoid factors and lung disease.
Br. J. Hosp. Med. 2, 507.

TURNER-WARWICK, M. (1972)
Cryptogenic fibrosing alveolitis.
Br. J. Hosp. Med. 7, 697.

TURNER-WARWICK, M. (1973)
Immunology and asbestosis.
Proc. R. Soc. Med. 66, 927.

TURNER-WARWICK, M. (1974a)
Immunological aspects of systemic diseases of the lungs.
Proc. R. Soc. Med. 67, 541.

TURNER-WARWICK, M. (1974b)
Auto antibodies in allergic respiratory disease.
Prog. Allergy 11, 283.

TURNER-WARWICK, M. & DONIACH,D. (1965)
Auto-antibody studies in interstitial pulmonary fibrosis.
Br. Med. J. 1, 886.

TURNER-WARWICK, M. & HASLAM, P. (1970)
Smooth muscle antibody in bronchial asthma.
Clin. Exp. Immunol. 7, 31.

TURNER-WARWICK, M. & HASLAM, P. (1971)
ANTIBODIES IN SOME CHRONIC FIBROSING LUNG DISEASES.
1. Non organ-specific autoantibodies.
Clin. Allergy 1, 83.

TURNER-WARWICK, M., HASLAM, P. & WEEKS, J. (1971)
Antibodies in some chronic fibrosing lung diseases.
11. Immunofluorescent studies.
Clin. Allergy 1, 209.

TURNER-WARWICK, M. & PARKES, W.R. (1970)
Circulating rheumatoid and antinuclear factors in asbestos workers.
Br. Med. J. 2, 492.

VAZQUEZ, J. J. & DIXON, F.J. (1957)
Immunohistochemical study of lesions in rheumatic fever, systemic lupus erythematosus and rheumatoid arthritis.
Lab. Invest. 6, 205.

VIGLIANI, E.C. (1968)
The fibrogenic response to asbestos.
Med. Lav. 59, 401.

VIGLIANI, E.C. & PERNIS, B. (1958)
Immunopathological factors in the pathogenesis of the hyaline tissue of silicosis.
Br. J. Ind. Med. 15, 8.

VIGLIANI, E. & PERNIS,B. (1959)
An immunological approach to silicosis.
In "Proceedings of the Pneumoconiosis Conference", Johannesburg.
Published by J. and A. Churchill, Ltd., London, p.395.

VIGLIANI, E.C., PERNIS, B. & MONACO, L. (1961)
A study on the action of quartz particles on macrophages in vitro.
In "Inhaled particles and vapours".
Published by Pergamon Press, p.348.

VOGT, V.M. (1973)
Purification and further properties of single-strand-specific nuclease from Aspergillus oryzae.
Eur. J. Biochem. 33, 192.

WAGNER, J.C. & McCORMICK, J.N. (1967)
Immunological investigations of coalworkers' disease.
J. R. Coll. Physicians Lond. 2, 49.

WALKER, J.G., DONIACH, D., ROITT, I.M. et al. (1965)
Serological tests in diagnosis of primary biliary cirrhosis.
Lancet 1, 827.

WARD, D.J., JOHNSON, G.D. & HOLBOROW, E.J. (1964)
Antinuclear factor in rheumatoid arthritis, its incidence and clinical significance.
Ann. Rheum. Dis. 23, 306.

WEAVER, A.L., BRUNDAGE, B.H., NELSON, R.A. et al. (1968)
Pulmonary involvement in polymyositis: report of a case with response to corticosteroid therapy.
Arthritis Rheum. 11, 765.

WEAVER, A.L., DIVERTIE, M.B. & TITUS, J.L. (1967)
The lung in scleroderma.
Mayo Clin. Proc. 42, 754.

WEBB, J., WHALEY, K. & LEE, P. (1974)
Clinical significance of native DNA antibodies in systemic lupus erythematosus and other connective tissue diseases.
Scott. Med. J. 19, 171.

WEBSTER, I. (1972)
Desquamative pneumonitis following A_Z Asian flu.
(Unpublished).

WEIGLE, W.O. (1973)
Immunological unresponsiveness.
In "Advances in Immunology".
Edited by F. J. Dixon and H. G. Kunkel.
Published by Academic Press. New York and London.
Volume 16. p.61-114.

WEIR, D.M. & HOLBOROW, E.J. (1962)
Serum anti-nuclear factor. Laboratory studies.
Ann. Rheum. Dis. 21, 40.

WELLER, T.H. & COONS, A.H. (1954)
Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro
Proc. Soc. Exp. Biol. 86, 789.

WHITEHOUSE, J.M.A. & HOLBOROW, E.J. (1971)
Smooth muscle antibody in malignant disease.
Br. Med. J. 4, 511.

WHITTINGHAM, S., IRWIN, J., MACKAY, I.R. et al. (1969)
Autoantibodies in healthy subjects.
Australas. Ann. Med. 18, 130.

WINCHESTER, R.J., AGNELLO, V. & KUNKEL, H.G. (1970)
Gammaglobulin complexes in synovial fluids in patients with rheumatoid arthritis.
Clin. Exp. Immunol. 6, 689.

WINFIELD, J.B. & DAVIS,J.S. (1974)
Anti-DNA antibody in procainamide-induced lupus erythematosus. Determinations using DNA fractionated by methylated albumin-kieselguhr chromatography.
Arthritis Rheum. 17, 97.

WOLD, R.T., YOUNG,F.E., TAN, E.M. et al. (1968)
Deoxyribonucleic acid antibody: a method to detect its primary interaction with deoxyribonucleic acid.
Science 161, 806.

ZAMENHOF, S. (1958)
Deoxyribonucleic acid (DNA).
In "Biochemical Preparations".
Edited by C. S. Vestling.
Published by John Wiley & Sons, New York.
Volume 6, p.8.

ZHDANOV, V.M. (1975)
Integration of viral genomes.
Nature 256, 471.

ACKNOWLEDGEMENTS

I wish to give most special thanks to Professor Margaret Turner-Warwick for allowing me the honour of working under her guidance; for all she has taught me, and for her patience, continual encouragement and kindness.

I also gratefully thank the many colleagues who have so willingly given me their help, in particular Miss Anna Lukoszek for her expert technical assistance; Miss Heather Rolls, Mrs. Virginia Bucknor and Miss Kathy Howarth for typing the manuscript; Miss Margaret Rehahn and Mr. Andrew Nunn for advice and teaching of statistical methods; Mr. Kenneth Moreman and Miss Milena Potucek for the photography; Miss Lindsey Pegas for the art work, and the physicians of the Brompton Hospital and Cardiothoracic Institute for allowing me to study their patients and for help with blood sampling. In addition, I wish to give special thanks to Dr. Ian Griffiths for conducting the Farr DNA-binding assays; Dr. E. W. Johns for providing the calf thymus deoxyribonucleoprotein; Dr. Robert Kamen for providing the Nuclease SI; Dr. Kenneth Shooter and Mr. Regis Picot for determining the physicochemical characteristics of the DNA preparations, and Miss Barbara Meyrick for providing the electron microcgraph.

Finally, I should like to gratefully acknowledge the sponsorship of the Medical Research Council, and give special thanks to my family who have helped in so many ways.

APPENDIX

STATISTICAL METHODS AND TABULATED RESULTS

STATISTICAL METHODS

Miss Margaret Rehahn, statistician to the Brompton Hospital and Cardiothoracic Institute, and Mr. Andrew Nunn, statistician of the M.R.C. Tuberculosis and Chest Diseases Research Unit very kindly advised on the following statistical methods.

METHOD 1 To test whether the proportion of occurrences of a single variable characteristic in two different populations was the same in both.

Statistical test: Test of difference of two proportions.

Reference: Hayslett, H.T. in "Statistics made simple" edited by P. Murphy, published by W. H. Allen 1968, p.122-124.

METHOD 2 To test the assumption that the two events occur with equal frequency within a single population.

Statistical test: Tested using the binomial distribution.

Reference: "Documenta Geigy Scientific Tables", edited by Konrad Diem, published by Geigy Pharmaceutical Co. Ltd. Manchester, 6th edition, p.105.

METHOD 3 To test for a significant association between two different variable characteristics in a single population (Bivariate distribution).

Statistical tests: a) Fisher fourfold table test for populations numbering 50 or less.

Reference: "Documenta Geigy Scientific Tables", edited by Konrad Diem, published by Geigy Pharmaceutical Co. Ltd. Manchester, 6th edition, p.109-123.

b) Chi square (X^2) test with Yates continuity correction, for populations numbering more than 50.

Reference: Siegel S. in "Non parametric statistics for the behavioural sciences", published by McGraw-Hill 1956, p.107.

METHOD 4 To test for differences in the mean values of quantitative test data from two different populations the conditions being that the data are normally distributed, that the variances are not significantly different, that the samples are random.

Statistical test: Student t-test (un-paired data).

Reference: Hayslett, H.T. in "Statistics made simple", edited by P. Murphy, published by W. H. Allen 1968, p.124-130.

Normalization of data: This test was used to compare ANA titre distributions which are extremely skew therefore it was necessary to normalize the data by logarithmic transformations, i.e. using the $\log_{10}$ of each reciprocal ANA titre reading (Reference: Lutz, W. "Statistical methods as applied to immunological data", in "Handbook of experimental immunology", edited by D. M. Weir, published by Blackwell 1967, p.1184-1191). The Variance ratio test (F-test) was used to confirm normalization of the data (Reference: Moroney, M.J. in "Facts from figures", published by Penguin Books 1973, p.233-236).

Method 5 To test whether variations in numerical values of two measurable random variables within a single population were independent or related.

Statistical test: Linear regression.

Reference: Hayslett, H.T. in "Statistics made simple", edited by P. Murphy, published by W. H. Allen 1968, p.131-134.

Hypothesis All statistical tests were used as two-tailed tests of the null hypothesis, and this was accepted for probability levels > 0.1.

STATISTICAL TABLE 1

(Supplement to Figure 2)

The probability of obtaining the observed differences in the prevalence of immunofluorescent ANA (titre ≥ 1 in 10) in normal controls of different ages by chance (Statistical method 1).

		Comparing ⟶ Age group:	30-39	40-49	50-59	60-69	70 & Over
		ANA Prevalence ↓ Age group:					
a.	Total Normal Controls	20-29	NS	0.05	0.05	0.02	0.001
		30-39		0.1	0.1	0.1	0.01
		40-49			NS	NS	NS
		50-59				NS	NS
		60-69					NS
b.	Males Only	20-29	NS	NS	0.1	0.02	NS
		30-39		NS	NS	0.05	NS
		40-49			NS	NS	NS
		50-59				NS	NS
		60-69					NS
c.	Females Only	20-29	NS	NS	NS	NS	0.01
		30-39		NS	NS	NS	0.1
		40-49			NS	NS	NS
		50-59				NS	NS
		60-69					0.05

NS = $p > 0.1$

STATISTICAL TABLE 2

(Supplement to Table 4)

The probability of obtaining the observed differences in the prevalence of immunofluorescent ANA (titre $\geq$ 1 in 10) in different lung diseases by chance (Statistical Method 1).

COMPARING ⟶ CFA	ASB	SIL	COAL	INT.	EXT.
ANA			PN	AST.	AST.
PREVALENCE ↓					
SLE 0.001	0.001	0.001	0.001	0.001	0.001
CFA	0.01	NS	0.01	0.01	0.01
ASB		0.1	NS	NS	NS
SIL			0.01	NS	0.01
COAL PN				NS	NS
INT. AST.					NS

NS = $P > 0.1$

STATISTICAL TABLE 3

(Supplement to Figure 3)

The probability of obtaining the observed differences in the prevalence of immunofluorescent ANA (titre $\geq$ 1 in 10) with age in patients with asbestosis by chance (Statistical method 1).

a. A comparison of the ANA prevalence in patients with asbestosis and in normal controls of similar age

COMPARING ⟶ Normal Controls			
ANA PREVALENCE ↓			
Asbestosis	Age:	20-29	NS
		30-39	NS
		40-49	NS
		50-59	NS
		60-69	0.01

b. A comparison of the ANA prevalence in asbestosis patients of different ages

COMPARING ⟶ Age group:			
ANA PREVALENCE ↓	40-49	50-59	60-69
Age group:			
30-39	NS	NS	0.1
40-49		NS	0.05
50-59			0.1

NS = $p > 0.1$

STATISTICAL TABLE 4

(Supplement to Table 7)

The probability of obtaining the observed differences in the distribution of positive titres of immunofluorescent ANA in the different lung diseases by chance (Statistical method 4).

COMPARING ⟶	CFA	Asbestosis	Silicosis
ANA titres ↓			
SLE	0.001	0.001	0.001
CFA		0.1	NS
Asbestosis			NS

NS = $p > 0.1$

STATISTICAL TABLE 5

(Supplement to Table 8)

The probability of obtaining the observed differences in the distribution of positive titres of immunofluorescent ANA in (a) females, (b) males with different lung diseases by chance (Statistical method 4).

a. Female patients only

COMPARING ⟶	CFA
ANA titres ↓	
SLE	0.05

b. Male patients only

COMPARING ⟶	CFA	Asbestosis	Silicosis
ANA titres ↓			
SLE	0.01	0.01	0.05
CFA		NS	NS

NS = $p > 0.1$

STATISTICAL TABLE 6

(Supplement to Table 9)

The probability of obtaining the observed differences in the proportions of IgG, IgM and IgA ANA in ANA positive sera in the different lung diseases by chance (Statistical method 1).

	COMPARING ANA Ig class frequencies ↓	CFA	ASB	SIL	INT AST
a. IgG ANA	SLE	0.05	0.01	NS	NS
	CFA		NS	NS	NS
	ASB			0.05	0.05
	SIL				NS
b. IgM ANA	SLE	NS	NS	0.02	NS
	CFA		NS	0.05	NS
	ASB			0.05	NS
	SIL				NS
C. IgA ANA	SLE	NS	NS	NS	NS
	CFA		NS	NS	NS
	ASB			NS	NS
	SIL				NS

NS = $p > 0.1$

STATISTICAL TABLE 7

(Supplement to Table 10)

The probability of obtaining the observed differences in the proportions of IgG, IgM and IgA ANA in male and female patients by chance (Statistical method 1).

		Males:				Females:	
	COMPARING ANA Ig class frequencies ↓	CFA	ASB	SIL	INT. AST	CFA	INT. AST
a. IgG ANA	SLE	NS	0.1	NS	NS	0.02	NS
	CFA		0.05	NS	NS		NS
	ASB			0.1	NS		
	SIL				NS		
b. IgM ANA	SLE	NS	NS	NS	NS	NS	NS
	CFA		NS	NS	NS		NS
	ASB			0.02	NS		
	SIL				NS		
c. IgA ANA	SLE	0.05	NS	NS	NS	0.1	NS
	CFA		0.05	0.1	0.1		NS
	ASB			NS	NS		
	SIL				NS		

d. COMPARING ANA Ig class frequencies →		Female patients: IgG ANA	IgM ANA	IgA ANA
Male patients:	SLE	NS	0.05	NS
	CFA	0.05	NS	NS

NS = $p > 0.1$

STATISTICAL TABLE 8

(Supplement to Figure 7)

The probability of obtaining the observed differences in the profiles of immunoglobulin classes of ANA in ANA positive sera in the different lung diseases by chance (Statistical method 1).

		COMPARING ⟶ Ig class profile ↓	CFA	ASB.	SIL.	INT. AST
a.	IgG ANA alone	SLE	NS	NS	0.01	NS
		CFA		NS	0.05	NS
		ASB.			0.05	NS
		SIL.				NS
b.	IgM ANA alone	SLE	0.05	0.01	NS	NS
		CFA		NS	NS	NS
		ASB.			0.05	0.05
		SIL.				NS
c.	IgG/IgM/IgA ANAs	SLE	NS	0.1	NS	NS
		CFA		0.1	NS	NS
		ASB.			NS	NS
		SIL.				NS
d.	IgG/IgM ANAs	SLE	0.05	0.1	0.05	NS
		CFA		NS	NS	0.05
		ASB.			NS	0.1
		SIL.				0.05

NS = $p > 0.1$

STATISTICAL TABLE 9

(Supplement to Figure 8)

The probability of obtaining the observed differences in the frequency of the various profiles of immunoglobulin classes of ANA in male and female patients by chance (Statistical method 1).

			Males:				Females:	
		COMPARING ⟶	CFA.	ASB.	SIL.	INT. AST	CFA	INT. AST
		Ig class profile ↓						
a.	IgG ANA alone	SLE	NS	NS	NS	NS	NS	NS
		CFA		NS	NS	NS		NS
		ASB.			0.02	NS		
		SIL.				NS		
b.	IgM ANA alone	SLE	NS	NS	NS	NS	0.02	NS
		CFA		0.05	NS	NS		NS
		ASB.			0.1	NS		
		SIL.				NS		
c.	IgG/IgM/IgA ANAs	SLE	0.05	NS	NS	NS	NS	NS
		CFA		0.02	0.1	0.1		NS
		ASB.			NS	NS		
		SIL.				NS		
d.	IgG/IgM ANAs	SLE	NS	NS	0.05	NS	NS	NS
		CFA		NS	NS	0.1		
		ASB.			NS	NS		
		SIL.				0.02		

NS = $p > 0.1$

STATISTICAL TABLE 10

(Supplement to Table 13)

The probability of obtaining the observed differences in the ANA titre distributions in sera with different profiles of immunoglobulin classes of ANA by chance (Statistical method 4).

COMPARING ANA titres →	IgM ANA alone	IgG/IgM/ IgA ANAs	IgG/IgM ANAs
IgG ANA alone	NS	0.01	NS
IgM ANA alone		0.01	0.01
IgG/IgM/IgA ANAs			0.01

NS = $p > 0.1$

STATISTICAL TABLE 11

(Supplement to Figure 9)

a. The probability of obtaining the observed differences in the frequencies of the various immunofluorescent staining patterns in ANA positive sera by chance (Statistical method 1).

COMPARING ⟶ / pattern frequencies ↓	Peripheral	Speckled	Nucleolar
Homogeneous	0.001	0.001	0.001
Peripheral		0.05	0.1
Speckled			NS

b. The probability of obtaining the observed differences in the frequency of occurrence of the various immunofluorescent staining patterns alone or in combination with other patterns by chance (Statistical method 2).

COMPARING ⟶ / frequency of occurrence ↓		In combination with other patterns
As a lone pattern :	Homogeneous	< 0.01
	Peripheral	NS
	Speckled	NS
	Nucleolar	< 0.01

NS = $p > 0.1$

STATISTICAL TABLE 12

(Supplement to Figure 10)

The probability of obtaining the observed differences in the frequency of the various immunofluorescent ANA staining patterns in ANA positive sera in the different lung diseases by chance (Statistical method 1).

		COMPARING ⟶ CFA ANA pattern frequencies ↓		ASB.	SIL.	INT. AST.
a.	Homogeneous pattern	SLE	NS	NS	NS	NS
		CFA		NS	NS	NS
		ASB.			NS	NS
		SIL.				NS
b.	Peripheral pattern	SLE	NS	NS	0.05	0.01
		CFA		NS	NS	0.1
		ASB.			NS	NS
		SIL.				NS
c.	Speckled pattern	SLE	NS	NS	0.05	NS
		CFA		NS	NS	NS
		ASB.			NS	NS
		SIL.				NS
d.	Nucleolar pattern	SLE	NS	0.05	NS	NS
		CFA		0.02	0.1	NS
		ASB.			NS	NS
		SIL.				NS

NS = $p > 0.1$

STATISTICAL TABLE 13

(Supplement to Figure 12)

The probability of obtaining the observed variability in the frequency of different immunofluorescent staining patterns in different immunoglobulin classes of ANA by chance (Statistical method 1).

		COMPARING ⟶ IgM ANA	IgA ANA
		pattern frequency ↓	
a.	Homogeneous pattern	IgG ANA	0.001	0.001
		IgM ANA		0.001
b.	Peripheral pattern	IgG ANA	0.01	0.02
		IgM ANA		0.001
c.	Speckled pattern	IgG ANA	0.1	NS
		IgM ANA		0.01
d.	Nucleolar pattern	IgG ANA	NS	NS
		IgM ANA		NS

NS = $p > 0.1$

STATISTICAL TABLE 14

(Supplement to Table 15)

The probability of obtaining the observed differences in the proportions of ANA positive sera with complement fixing ANA in the different lung diseases by chance (Statistical method 1).

COMPARING ⟶ / C^1 fixing ANA frequency ↓	CFA	ASB.	SIL.	INT. AST.
SLE	NS	0.001	0.05	0.05
CFA		0.01	0.1	0.05
ASB.			0.1	NS
SIL.				NS

NS = $p > 0.1$

STATISTICAL TABLE 15

(Supplement to Table 16)

The probability of obtaining the observed differences in the proportion of sera with complement fixing ANA in male and female patients by chance (Statistical method 1).

a. COMPARING ——————————→ Female patients

C^1 fixing ANA frequency

↓

Male patients:		
	SLE	NS
	CFA	NS

b.

		Males:				Females:	
COMPARING ——→	CFA	ASB.	SIL.	INT. AST	CFA	INT. AST
C^1 fixing ANA frequency ↓						
SLE	NS	NS	NS	NS	NS	0.1
CFA		0.05	NS	NS		NS
ASB.			0.1	NS		
SIL.				NS		

NS = $p > 0.1$

STATISTICAL TABLE 16

(Supplement to Figure 14)

The probability of obtaining the observed differences in the frequency of the various immunoglobulin class profiles of ANA in sera with and without complement fixing ANA by chance (Statistical method 1),

COMPARING → Ig class profile ↓		Sera without C^1 fixing ANA
Sera with C^1 fixing ANA :	IgG ANA alone	NS
	IgM ANA alone	0.05
	IgG/IgM/IgA ANAs	0.01
	IgG/IgM ANAs	NS

NS = $p > 0.1$

STATISTICAL TABLE 17

(Supplement to Table 19)

The probability of obtaining the observed differences in the patterns of ANA immunofluorescence in sera with and without complement fixing ANA by chance (Statistical method 1).

a. COMPARING ANA pattern frequency →		Sera without C^1 fixing ANA
Sera with C^1 fixing ANA	Homogeneous	NS
	Peripheral	0.05
	Speckled	0.001
	Nucleolar	0.001

b. COMPARING ANA pattern frequency →		Sera without C^1 fixing ANA
Sera with C^1 fixing ANA	Lone homogeneous	0.01
	Homogeneous in pattern combinations	0.1

NS = $p > 0.1$

STATISTICAL TABLE 18

(Supplement to Table 20)

The probability of obtaining the observed differences in the frequency of antibodies to deoxyribonucleoprotein (NP) in ANA positive sera in the different lung diseases by chance (Statistical method 1).

COMPARING ⟶	CFA	ASB.	SIL.	INT. AST
NP antibody frequency ↓				
SLE	0.001	0.001	0.001	0.01
CFA		NS	NS	NS
ASB.			NS	NS
SIL.				NS

NS = $p > 0.1$

STATISTICAL TABLE 19

(Supplement to Table 21)

The probability of obtaining the observed differences in the frequency of antibodies to deoxyribonucleoprotein (NP) in male and female patients by chance (Statistical method 1).

	Males:				Females:	
COMPARING ——————→	CFA	ASB.	SIL.	INT. AST	CFA	INT. AST
NP antibody frequency ↓						
SLE	0.01	0.01	0.01	0.05	0.01	NS
CFA		NS	NS	NS		NS
ASB.			NS	NS		
SIL.				NS		

NS = $p > 0.1$

STATISTICAL TABLE 20

(Supplement to Table 27)

The probability of obtaining the observed differences in the frequency of Ouchterlony precipitins to Nuclease S1-treated calf thymus 'DS'-DNA in ANA positive sera in the different lung diseases by chance (Statistical method 1).

COMPARING ⟶	CFA	ASB.	SIL.	INT.
'DS'-DNA				AST
precipitin frequency ↓				
SLE	NS	NS	0.1	0.05
CFA		0.05	NS	0.02
ASB.			0.01	NS
SIL.				0.01

NS = $p > 0.1$

STATISTICAL TABLE 21

(Supplement to Table 28)

The probability of obtaining the observed differences in the frequency of Ouchterlony precipitins to Nuclease S1-treated calf thymus 'DS'-DNA in male patients by chance (Statistical method 1).

COMPARING ⟶ Male patients:					
'DS'-DNA precipitin frequency ↓		CFA	ASB.	SIL.	INT. AST
Male patients:	SLE	NS	NS	0.05	NS
	CFA		NS	NS	NS
	ASB.			0.01	NS
	SIL.				0.05

NS = $p > 0.1$

STATISTICAL TABLE 22

(Supplement to Table 34)

The probability of obtaining the observed differences in the frequency of Ouchterlony precipitins to calf thymus SS-DNA in ANA positive sera in the different lung diseases by chance (Statistical method 1).

COMPARING ⟶	CFA	ASB.	SIL.	INT. AST
SS-DNA precipitin frequency ↓				
SLE	0.1	0.01	NS	0.02
CFA		NS	NS	NS
ASB.			NS	NS
SIL.				NS

NS = $p > 0.1$

STATISTICAL TABLE 23

(Supplement to Table 35)

The probability of obtaining the observed differences in the frequency of Ouchterlony precipitins to calf thymus SS-DNA in male and female patients by chance (Statistical method 1).

COMPARING ⟵		⟶ Female patients
SS-DNA precipitin frequency ↓		
Male patients	SLE	0.1
	CFA	0.05
	Total patients	0.001

NS = $p > 0.1$

STATISTICAL TABLE 24

(Supplement to Table 42)

The probability of obtaining the observed differences in the frequency of positive Farr-globulin binding with I^{125}-HeLa cell DS-DNA in positive sera in the different lung diseases by chance (Statistical method 1).

COMPARING ⟶	CFA	ASB.	SIL.	INT. AST.
DS-DNA binding antibody frequency ↓				
SLE	0.05	0.01	NS	NS
CFA		NS	NS	NS
ASB.			NS	NS
SIL.				NS

NS = $p > 0.1$