Cytodiagnosis of rheumatoid pleural effusions

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SYNOPSIS The stained smears of the deposits from one pericardial and 19 pleural effusions complicating rheumatoid arthritis were examined. On the basis of clinical and biochemical evidence it was considered that in six cases the effusions were due to the rheumatoid disease while in a further nine cases the association was considered likely. In the remaining five cases the association was considered to be due to chance as other causes for the effusions were diagnosed.

On cytological examination, seven cases showed a characteristic picture of degenerating polymorphs with amorphous extracellular material and epithelioid cells many of which were multinucleate. Five others contained similar amorphous material without epithelioid cells; of these two had many plasma cells and a third numerous macrophages probably containing 'droplets' of rheumatoid factor complex.

Thus in 12 of 15 cases a definite diagnosis of rheumatoid effusion could be made. In the remaining five cases cytological examination confirmed that the effusions were unrelated to the rheumatoid disease.

The extracellular material gave a non-specific fluorescence with labelled anti- γ globulin antisera, and since this reaction was not seen in control pleural fluid deposits, or with preparations of fibrin, it may have a confirmatory value.

It is concluded that in many cases reliable cytological evidence can be found to confirm or refute a diagnosis of rheumatoid pleural or pericardial effusion. This may be helpful in the management of the rheumatoid disease.

The pleura and lungs may be involved in rheumatoid disease and an increased incidence of pleurisy, pleural effusion, and diffuse interstitial fibrosis in patients with rheumatoid arthritis compared with controls has been recorded by several authors (Walker and Wright, 1967 and 1968; Turner-Warwick, 1969). The frequency of otherwise unexplained pleural effusion attributed to rheumatoid disease is given by Walker and Wright (1967) as 3.3% of patients with rheumatoid arthritis (7.9% in males and 1.6% in females). Rheumatoid pleural effusions are frequently symptomless; they are often persistent, responding poorly to treatment, but rarely give rise to complications.

Pericardial involvement by rheumatoid disease is also well documented (Harrold, 1968) but in contrast to pleural involvement the sequelae, which include constrictive pericarditis, may be more serious.

Patients with rheumatoid arthritis may have

pleural or pericardial involvement due to other causes, and the establishment of an association between the arthritis and the pleural or pericardial disease remains difficult (*British Medical Journal*, 1967). It is therefore valuable to have methods of identifying the effusions which are of rheumatoid origin.

Until recently it was not realized that there was any morphological picture characteristic of rheumatoid effusion. Leucocytes containing inclusions have been described, but these 'ragocytes' (Delbarre, Kahan, Amor, and Krassanine, 1964) or 'R.A. cells' (Hollander, McCarty, Astorga, and Castro-Murillo, 1965), originally seen in rheumatoid synovial fluid, are not specific for the disease. In 1968, Nosanchuk and Naylor published a description of 'a unique cytologic picture' in rheumatoid pleural effusions. This comprised two main components: (1) a background of amorphous material, and (2) the presence of large epithelioid cells, often elongated or with tails, and showing multinucleation.

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Soon after the appearance of this paper we encountered several similar cases. This led us to search the files for previous smears from serous effusions complicating rheumatoid arthritis, and the series so formed has enabled us to form a clearer picture of the various cytological appearances. These are by no means uniform, but are often characteristic enough to be of diagnostic help. We have also attempted in the more recent cases to identify immune complexes inside and outside the cells, using immunological techniques.

Material and Methods

The series comprises 20 patients with definite rheumatoid arthritis according to the diagnostic criteria of the American Rheumatism Association (1959). Nineteen of these patients had pleural effusions and one a pericardial effusion. Several of these patients were admitted to hospital a number of years ago and in some cases only limited laboratory findings on the effusions were available. The clinical records of all these patients have been studied, and on the basis of the available evidence, excluding the cytological appearances of the fluid, the effusions have been classified as being (a) due to rheumatoid disease, (b) probably due to rheumatoid disease, and (c) due to other diseases (Table I). In 13 cases only the smears (stained with May-Grünwald and Giemsa, and in some cases also with periodic acid-Schiff and by Papanicolaou's method) were avail-

able. In seven cases (all involving the pleura) it was possible to perform other tests.

For immunofluorescence, the fluids were centrifuged and the deposits washed with three changes of 10% normal rabbit serum or 2% bovine albumin in saline. Smears of the deposit were then treated as described by Solomon, Fahey, and Malmgren (1963), but stained for 30 minutes and mounted using 75% glycerol. Fluorescent antisera were supplied by Hyland laboratories.

Agar diffusion was carried out by the Ouchterlony-Elek plate method using phosphate-buffered agar.

Rheumatoid factor was detected in fluids, after removal of deposit, by the R.A. latex method (Mercia diagnostics).

Immunoglobulin levels were carried out by the immunoplate method (Hyland).

For electron microscopy the deposit was fixed in phosphate buffered 1% OsO_4 , pH 7.4. Sections were stained with 2% aqueous uranyl acetate followed by lead citrate. They were examined with a Philips EM100 at 60 kV.

Results

CYTOMORPHOLOGY

Table I lists the cases in our series, 19 with pleural and one with a pericardial effusion. Besides the clinical classification, three features of the smears are given (degenerate polymorphs, amorphous extracellular material, and epithelioid cells). These

Case	Sex	Age	Interval between Arthritis and Effusion	Classification				Final
				Clinical	Cytological			
					Degenerative Polymorphs	Amorphous Material	Epithelioid Cells	
1	F	71	18 months	a	+	+	+	a
2	F	58	6 years	a	+	+	+	a
3	M	42	Effusion first	a	+	+	+	a
4	M	60	10 years	a	+	+	+	a
5	F	62	3 years	a	+	+	+	a
6	F	80	1 year	a	-	+	-	a
7	M	45	3 years	b	+	+	+	a
8	F	79	Many years	b	+	+	+	a
9	M	54	8 years	b	+	+	+	a
10	F	78	21 years	b	-	+	+	a
11	M	64	8 years	b	+	+	-	a
12 ¹	M	47	3 years	b	-	+	-	a
13	F	64	2 years	b	-	-	-	b
14	M	56	2 months	b	-	-	-	b
15	F	70	6 years	b	-	-	-	b
16	M	66	13 years	c	-	-	-	c
17	F	65	13 years	c	-	-	-	c
18	M	59	18 years	c	-	-	-	c
19	M	60	6 months	c	-	-	-	c
20	M	60	20 years	c	-	-	-	c

Table I Classification of effusions

¹Pericardial effusion. a = due to rheumatoid disease, b = probably due to rheumatoid disease, c = due to other causes

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are chosen as the cardinal features of the fully developed rheumatoid effusion. Seven cases (1, 2, 3, 4, 7, 8, and 9) were seen with the complete picture, as described by Nosanchuk and Naylor (1968), and five others (cases 5, 6, 10, 11, and 12) showed partially developed or variant forms as described below. There were three cases of probable rheumatoid pleural effusion in which the cytological picture was entirely non-specific (cases 13, 14, and 15). The remaining five cases (cases 16-20), classified as 'c' in Table I, had a coincident pleural effusion unrelated to the rheumatoid arthritis, and the cytological picture was irrelevant.

Sterile pleural effusions generally contain a high proportion of well preserved living cells, and debris is taken up by macrophages (Spriggs and Boddington, 1968). However, the first impression of the typical rheumatoid pleural fluid deposit is its resemblance to that of an empyema. In many of these effusions

the stained films show a background of amorphous material and abundant necrotic leucocytes. But whereas ordinary pus consists mainly of dying neutrophils, the rheumatoid exudate also contains many mononuclear cells. Bare nuclei are common, sometimes losing their chromatin pattern and becoming hyaline. In addition, there are macrophages in varying numbers, and some of these produce bizarre giant epithelioid forms, some of them multinucleate or with a large irregular nucleus. These epithelioid cells often take on a tadpole shape, with a pointed tail—a configuration practically unknown in other cells in effusions. These appearances are illustrated in Figs. 1 and 2 from case 3. In an experience of 2,800 pleural effusions we have never seen this picture in any other condition than rheumatoid arthritis.

The amorphous background material is variable in its staining characteristics, mostly being baso-

Fig. 1. Case 3. Pleural fluid deposit, showing a background of degenerating polymorphs and mononuclear cells, and two multinucleate giant cells of epithelioid type. Both the examples shown have well developed tails of cytoplasm. May-Grünwald and Giemsa, $\times 340$.

Fig. 2. Case 3. Another field at the same magnification, but a wet-fixed smear stained by Papanicolaou's method. This also shows two epithelioid cells, the larger one having a long tail of cytoplasm.

philic but sometimes eosinophilic. In cases 3, 5, 7, and 10 there were clusters of discrete spherical structures about 2 to 3 μ across, staining green in Papanicolaou-stained smears and grey-blue with Romanowsky stains; cases 4, 8, 9, and 11 (Fig. 6) contained fused lumps of hyaline material and in cases 1 and 2 there were large masses staining pale blue or purple with Romanowsky stains, and (case 2) orange in Papanicolaou smears. The hyaline material is PAS positive, persisting after salivary digestion (Fig. 7). Fibrin can have similar staining characteristics but occurs in shreds rather than in lumps.

One of the effusions also contained abundant cholesterol crystals (case 4), and this probably represents the final stage in an effusion which is not absorbed (Ferguson, 1966; Walker and Wright, 1968; Stengel, Watson, and Darling, 1966).

Fig. 3. Case 6. Pleural fluid deposit. Top left of centre is a small mesothelial cell. Apart from some lymphocytes, the remaining cells are macrophages of various sizes. They have foamy cytoplasm containing unstained rounded vacuoles, corresponding to the protein demonstrated in Figures 11 and 12. May-Grünwald and Giemsa, $\times 760$.

In case 6 there was much amorphous material staining bluish in Romanowsky smears, orange with Papanicolaou, and strongly PAS positive; but instead of degenerating leucocytes with a predominance of polymorphs, the nucleated cells were well preserved and consisted mainly of macrophages. These cells were vacuolated and at first sight did not look unusual, but some of the spherical vacuoles had a hyaline appearance, and contained strongly PAS-positive material in the form of rounded droplets (Figs. 3, 8). In electron micrographs (Figs. 4, 5) these droplets were seen as almost spherical vacuoles, each surrounded by a unit membrane; the vacuoles contained finely granular material of low density arranged in a vaguely reticular pattern.

Another striking picture was presented by case 12, with a pericardial effusion. This fluid contained numerous red cells, lymphocytes, plasma cells, and some neutrophils. Among the plasma cells were many 'Mott cells' (Russell body cells). In addition, there were many clusters of hyaline globules, about the same size as the lymphocytes, and very faintly blue staining in the Romanowsky smear (Fig. 9). These show in thin parts of the smear as empty rounded areas. (A high-power field showing plasma cells from this case is Fig. 22 of Spriggs and Boddington (1968), but the extracellular globules are not shown.) A similar picture to this was seen in case 10.

Of the three cases with probable rheumatoid pleural effusion but no cytological confirmation, cases 13 and 14 showed a lymphocytic picture like that of a tuberculous effusion, and case 15 had a moderate pleural eosinophilia.

Of the five cases in which the pleural effusion was believed to be an incidental finding, cases 17 and 20 had purulent fluids; case 19 had a deposit with numerous mesothelial cells resulting from congestive cardiac failure; and cases 16 and 18 had malignant effusions due respectively to carcinoma of the lung and to an unknown primary, both full of malignant cells.

It will be seen from Table I that the cytological findings corroborate all the definite clinical diagnoses, and confirm the probable ones in six cases out of nine.

IMMUNOFLUORESCENCE STUDIES

Fluorescent anti- γ G, γ A, and γ M were applied to the deposit from cases 3, 4, 5, 6, and 11. The most remarkable feature of these five samples was the brilliant fluorescent staining of the aggregated material as shown in Figure 10. The intracellular material seen in case 6 also showed positive fluorescence (Fig. 11).

Fig. 4. *Case 6. Pleural fluid deposit. Electron micrograph showing a macrophage (and parts of others) with round vacuoles, corresponding to those seen in Figure 3. The surrounding pseudopodia are those of macrophages engaged in pinocytosis. $\times 11,200$.*

Fig. 5. Case 6. A higher magnification of another macrophage from the same section, showing the membrane-bounded vacuoles containing flocculent finely granular material. Glycogen is also abundant. $\times 39,700$.

Fig. 6. Case 11. Pleural fluid deposit, wet-fixed and stained Papanicolaou. Most of the cells are degenerating neutrophils and macrophages. Irregular masses of amorphous material are also seen, staining orange except at the periphery. $\times 240$.

Fig. 7. Case 11. Same material as in Figure 4, but air dried and stained with PAS. The amorphous material stains bright pink. $\times 240$.

Fig. 8. Case 6. Same material as in Figures 3, 4, and 5. Macrophages are seen containing rounded inclusions which are PAS-positive. $\times 540$.

Fig. 9. Case 12. Pericardial fluid deposit. Abundant red cells with lymphocytes and two plasma cells, one of 'Mott cell' type. Round unstained structures, many of them hardly larger than the lymphocytes, are scattered alone and in small groups, and are most obvious on the right where they aggregate to form larger masses. May-Grünwald and Giemsa, $\times 240$.

Fig. 10. Case 3. Washed pleural fluid deposit treated with fluorescein-labelled anti- γ M. Cells show pale blue autofluorescence. Amorphous material shows green fluorescence. $\times 240$.

Fig. 11. Case 6. Washed pleural fluid deposit (same as that of Figs. 3, 4, 5, 8, and 12) treated with fluorescein-labelled anti- γ M. The material in the vacuoles of macrophages contains γ globulin, fluorescing green. $\times 1,400$.

In 23 control fluids (17 pleural, 5 peritoneal, 1 pericardial) faint fluorescence, usually with anti- γ M, was seen intracellularly in seven cases; also in case 19 with congestive cardiac failure together with rheumatoid arthritis. Occasional fluorescing plasma cells were seen in the non-rheumatoid fluids and in case 20, but none were seen in the fluids of the rheumatoid effusions. Fluorescent extracellular material was not seen in any of the controls, and tests with fibrin from pleural fluid collected in a similar way to the other samples also gave an entirely negative result with anti- γ M and anti- γ G.

PRECIPITIN REACTIONS IN AGAR

The aggregated material from two patients (cases 4 and 6) was separated from the cellular deposit by centrifuging on an albumin gradient. After washing twice with saline the material was extracted with approximately three times its volume of glycine buffer, pH 3.4, at 37°C for 30 minutes, and after neutralizing with N/1 NaOH was tested against anti- γ G, γ A, γ M, and normal rabbit serum by agar diffusion. The second washing solution was also tested to ensure that soluble proteins had been removed. Both patients gave a strong precipitin line with anti- γ G, and case 19 gave a weaker line with anti- γ M (Fig. 12). No precipitin lines were obtained with anti- γ A or with normal rabbit serum.

Fig. 12. Case 6. Agar diffusion plate, on which the acellular part of the pleural fluid deposit was tested against antiglobulin sera. A strong precipitin line is shown with anti- γ G, and a weaker one with anti- γ M.

R.A. LATEX TEST

The results of testing dilutions of pleural fluids from 24 non-rheumatoid patients and four rheumatoid patients (cases 4, 5, 6, and 11) and the solutions of aggregated material from two, as used for the precipitin tests, are shown in Table II.

In case 6 the precipitate showed similar activity to the supernatant fluid. The supernatant from case 4 showed rheumatoid factor activity but the solutions of aggregated material did not.

Of 24 non-rheumatoid fluids, only three showed low levels of rheumatoid factor (++ at 1 in 20). One of these was from a case of systemic lupus erythematosus with L.E. cells in the fluid, one due to renal failure, and one of unknown cause in a child.

IMMUNOGLOBULIN LEVELS

The immunoglobulin levels in the effusion fluids of cases 4, 5, 6, and 11 and of 10 controls are shown in Table III. Although the highest levels of γ G and

	Dilution				
	1/20	1/40	1/80	1/160	1/320
Case 4					
Supernatant	++	++	-	-	-
Deposit	-	-	-	-	-
Case 5					
Supernatant	+++	+	+		
Case 6					
Supernatant	+++	++	+	-	
Deposit	++	+	-	-	
Case 11					
Supernatant	+++	+++	+++	++	+

Table II Latex R.A. tests on pleural fluids and on solutions of aggregated material¹

¹In 24 control pleural fluids, ++ reaction at 1/20 was found in three (D.L.E., renal failure, and cause unknown).

Case No.	Immunoglobulin Level (mg/100 ml)		
	γ G	γ A	γ M
<i>Rheumatoid Arthritis</i>			
4	1,230	205	13
5	900	360	150
6	830	195	60
11	1,170	360	111
<i>Controls</i>			
4212	700	285	23
4216	470	340	37
4282	660	250	23
4350	675	159	32
4370	960	435	72
4373	1,020	360	72
4375	900	330	60
4378	735	270	16
4379	675	99	27
4381	780	210	37

Table III Immunoglobulin levels in pleural fluids

γ M were in rheumatoid effusions, the differences are neither consistent nor very large.

Discussion

DIAGNOSTIC FEATURES OF RHEUMATOID EFFUSION

Contrary to expectation, rheumatoid pleural and pericardial effusions are not found in patients who have arthritis of great severity or of long duration, but in patients with variable disease histories (Walker and Wright, 1967). In our case 3 the effusion developed two months before the arthritis; this has been recorded by others (Carr and Mayne, 1962; Walker and Wright, 1968; Turner-Warwick, 1969) and adds to the diagnostic difficulties.

The establishment of rheumatoid disease as the cause of an effusion tends to be by exclusion of other causes, but various characteristics of the patients and their effusions have been described. These include a higher incidence in males than would be expected by the sex incidence of rheumatoid arthritis (which this series confirms); a higher incidence of positive serological tests for rheumatoid factor and rheumatoid nodules than in the general rheumatoid population; and an effusion protein content exceeding 3.0 g% indicating an exudate (Walker and Wright, 1967 and 1968). A fluid glucose level of less than 30 mg% in the absence of infection has been considered of diagnostic significance (Carr and Mayne, 1962; Campbell and Ferrington, 1968). Some authors have emphasized that very high values of lactic dehydrogenase in the fluid are almost diagnostic of a rheumatoid effusion (Berger and Seckler, 1966; Stengel *et al.*, 1966), while others doubt the significance of these findings since raised values are found in many conditions, especially tuberculosis and malignancy (Raabo, Rasmussen, and Terkildsen, 1966; Holten, 1968). Positive serological tests for rheumatoid factor in the pleural fluid, often to a higher titre than in the serum, have been used as a diagnostic aid (Walker and Wright, 1967), and although our results shows that the highest titres occurred in rheumatoid arthritis the finding of positive tests in other kinds of effusions, albeit at lower levels than is usual in rheumatoid arthritis, reduces the discriminating value of this test. Levine, Szanto, Griebble, Bach, and Anderson (1968) reported similar results and seven of their 65 fluids tested showed higher concentrations than in the serum.

Pleural biopsy sometimes provides a characteristic picture, with palisading of epithelioid cells. According to Champion, Robertson, and Robinson (1968) the appearance resembles 'a rheumatoid nodule opened out, exposing the fibrinoid zone to the

serous cavity'. Unfortunately this picture is not seen regularly enough for biopsy to be a reliable diagnostic test.

CYTOLOGICAL FINDINGS

In the past, little value has been attached to the cytological examination of the pleural fluid in rheumatoid arthritis. Mainly because most of these effusions were explained away as due to associated conditions, the occurrence of a distinctive picture was never noticed, either by us or by others with a large experience of the cells of effusions. Our findings on review fully confirm the discovery of Nosanchuk and Naylor (1968) that in certain cases the stained smears furnish morphological evidence of the rheumatoid process.

The combination of amorphous protein in the background with degenerating leucocytes and multinucleate epithelioid cells was seen in seven of our 20 cases. This is no doubt a reflection of the typical histological picture described by Champion *et al.* (1968). The multinucleate cells are not the same as those described by Wihman (1948) in allergic syndromes, of which 'rheumatic pleurisy' was one; in his cases clumps of mesothelial cells and multinucleates were associated with many eosinophils. In such cases the mesothelial cells have basophilic cytoplasm and they and the associated leucocytes are well preserved, whereas in our cases of rheumatoid arthritis the 'epithelioid cells' have less cytoplasmic basophilia, their nuclei and cytoplasm show degenerative changes, and the associated leucocytes also have a necrotic appearance. Even without the epithelioid cells, the appearance of the neutrophils and the extracellular material is fairly distinctive (cases 5 and 11).

A rather different, though characteristic, picture was seen in three other cases in our series; amorphous deposit was associated in two with frequent plasma cells (cases 10 and 12) and in the other with macrophages probably containing rheumatoid factor complex (case 6). The latter may be regarded as showing a form of the 'R.A. cell' phenomenon, to be described below.

The cytological appearances in six cases confirmed the diagnosis of 'definite' rheumatoid effusion made on the basis of the other clinical and pathological findings. In six further cases the cytological appearances allow a change from 'probable' to 'definite' to be made in the diagnostic labelling, while cytological examination does not alter the labelling of three other 'probable' rheumatoid arthritis effusions.

Thus the cytological picture described here, taken in conjunction with the other characteristics mentioned earlier, should often enable a diagnosis of

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rheumatoid effusion to be made more confidently; in its fully developed form it can be considered practically specific for the disease.

R.A. CELLS

There have been many descriptions of 'ragocytes' or 'R.A. cells' found in joint fluids in cases of rheumatoid arthritis (Delbarre *et al*, 1964; Delbarre, Amor, Kahan, and Krassamine, 1966; Astorga and Bollet, 1965; Hollander *et al*, 1965) and in rheumatoid pleural fluids (Berger and Seckler, 1966; Carmichael and Golding, 1967). These cells are usually neutrophils; they contain spherical bodies which are visible in fresh unstained preparations and give the staining reactions for neutral fat. Since neutrophils containing fat droplets are common in effusions of many different types, there is nothing very specific about this. Inclusions have been noted in peripheral blood and joint fluid leucocytes in nonrheumatoid patients (Vaughan, Barnett, Sobel, and Jacox, 1968a) and Astorga and Bollet (1965) found them in 20% of joint fluids from other kinds of arthritis so that as a morphological entity the R.A. cell hardly seems to deserve a special name.

The characteristic feature of these cells as originally described is that when washed and ruptured they release rheumatoid factor into the supernatant fluid, though this is not specific (Sones, McDuffie, and Hunder, 1968). Also inclusions have been found to show up with fluorescent anti- γ M (Delbarre *et al*, 1966), and fluorescent anti- γ G, γ A, and γ M (Vaughan *et al*, 1968a). These authors noted similar, but less marked, fluorescent reactions in the cells of non-rheumatoid cases, and this has been confirmed in this series. It looks, therefore, as if the neutrophils have taken up immune complexes. Electron micrographs show some, at least, of the inclusions to be phagolysosomes (Delbarre *et al*, 1966; Coimbra and Lopes-Vaz, 1967), and it is suggested that the R.A. cell is one which has scavenged a complex of rheumatoid factor with 7S globulin or other protein material. The relation between this and the Sudan-positive spherules is not clear; Lopes-Vaz and Coimbra (1967) have shown that the 'inclusion cells' of rheumatoid joints contain a number of different sorts of inclusions such as glycogen, fat, lysosomes, and multivesicular bodies as well as dense bodies which may be the immune complexes. They identified acid phosphatase in the phagolysosomes and in the dense bodies. These findings indicate that leucocyte or other cellular inclusions containing various globulins and other protein material are not specific for rheumatoid arthritis, but they do not detract from the possible pathogenetic significance of this cell in rheumatoid arthritis as discussed by Vaughan, Jacox, and Noell (1968b).

Certain other changes have been described in the leucocytes in synovial fluid in rheumatoid arthritis, such as nuclear fragmentation and extracellular particles of chromatin (Malinin, Pekin, and Zvaifler, 1967), but the description and illustrations do not suggest any really diagnostic feature.

IMMUNOFLUORESCENCE

In this series, fluorescent antisera were applied in five cases (3, 4, 5, 6, and 11), and all showed bright fluorescence of extracellular material with one or all of the antisera used (anti- γ G, γ A and γ M). It would be unjustifiable to draw conclusions about the composition of the extracellular material from these tests, because absorption studies lead us to regard much of the fluorescence as non-specific. Moreover, the substance probably varies greatly in composition, as it does in morphology. However, the reaction was not given by fibrin, which is the only similar material seen in smears of serous fluid deposits, and this gives the anti- γ globulin tests an empirical value which may well be helpful in cases of doubt. The fact that no fluorescent background material was seen in 23 deposits of pleural, peritoneal, and pericardial fluids of varying cause lends support to this.

Intracellular fluorescent material was seen, especially with anti- γ M, in seven of the controls as well as in case 6, and in two of the associated effusions in cases of rheumatoid arthritis (cases 19 and 20). The cells concerned were usually polymorphs or plasma cells but were sometimes unidentified. A positive result of this kind cannot therefore be regarded as good evidence of the disease.

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