Laboratory diagnosis of rheumatoid arthritis

Prospective study of 85 patients*

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Bayliss, C. E., Dawkins, R. L., Cullity, G., Davis, R. E., and Houlston, J. B. (1975). *Annals of the Rheumatic Diseases*, 34, 395–402. **Laboratory diagnosis of rheumatoid arthritis. Prospective study of 85 patients.** Because early diagnosis of rheumatoid arthritis is difficult but increasingly important, we have assessed the value of laboratory investigations in 85 patients with knee effusions studied from presentation and followed for sufficiently long periods to allow a definite diagnosis.

Histopathology on needle biopsy specimens narrowed the differential diagnosis to rheumatoid arthritis and closely related conditions even at an early stage of disease and also allowed recognition of other conditions which would not otherwise have been detected. Immunofluorescence on similar specimens further narrowed differential diagnosis since the presence of IgM was found to be very suggestive of rheumatoid arthritis. Other tests were of less value.

It is concluded that laboratory investigation can improve diagnostic sensitivity and specificity in relatively early rheumatoid arthritis.

Many cases of inflammatory joint disease must be classified initially as undifferentiated polysynovitis and months or years may elapse before specific diagnosis is possible. Diagnosis of rheumatoid arthritis (RA) on the basis of nonspecific, early manifestations often requires later reclassification of some cases. This situation is unsatisfactory, especially now that the use of immunosuppressive therapy is being considered early in the course of RA.

Since the report of Polley and Bickel (1951) several workers have assessed the value of synovial biopsy using a punch instrument (Rodnan, Yunis, and Totten, 1960; Mikkelsen, Duff, Castor, Zevely, and French, 1958) or needle (Parker and Pearson, 1963; Caughey and Highton, 1965; Wilkinson and Jones, 1963; Schumacher and Kulka, 1972). When the clinical diagnosis has been indeterminate, a needle biopsy has been informative in only 30–50% of cases, and it has been consistently noted that the specificity of histopathological changes has been particularly poor in RA. Immunofluorescence of synovial cells (Kinsella, Baum, and Ziff, 1969; Hurd, Kinsella, and Ziff, 1971) or of intact synovial membrane (Tursi, Trizio, and Bonomo, 1970) may show changes, but

whether this technique can be diagnostically helpful remains to be seen. Examination of joint fluid for rheumatoid factor, RA cells, neutrophil granulocytes, and complement has been widely used, but the results have given limited information.

We have assessed the diagnostic value of these methods when performed concurrently on patients who were then followed up for sufficiently long periods to allow specific diagnosis. We conclude that some of these tests are valuable.

Materials and methods

SUBJECTS AND DESIGN OF STUDY

Ninety-three consecutive patients with knee effusions were studied after referral for rheumatological consultation. There were no selection criteria other than a history of pain or swelling in one or more joints and the presence of an effusion in one knee. 73 subjects were referred because of difficult diagnosis or because of some atypical feature which suggested that two unrelated conditions might be present. The remaining 20 patients had been referred for long-term management. None of the group of 93 patients had nodules, vasculitis, or other extra-articular manifestations of rheumatoid disease. 85 patients provided

adequate data, including 39 males and 46 females ranging in age from 13 to 81 years.

On the basis of physical and x-ray examination before biopsy, patients were classified according to the criteria of the American Rheumatism Association (ARA) (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958), with the exception that criteria for exclusion of a diagnosis of RA were ignored. After biopsy patients were reviewed at intervals over a period of up to 3 years and reclassified according to ARA criteria when indicated by changes in the clinical state, but the results of the various investigations recorded here were not taken into account.

Immediately before biopsy synovial fluid was aspirated and specimens collected for white cell counts, estimation of complement activity, and titre of rheumatoid factor and examination for crystals and bacteria. Venous blood was also collected for estimation of complement and rheumatoid factor. The aspirated knee was then distended with normal saline and the Parker-Pearson biopsy needle inserted into the suprapatellar pouch under local anaesthesia. 5 to 6 specimens were then taken. 2 or 3 were snap frozen for immunofluorescence and the remainder fixed for routine histopathological examination. There were no complications.

CELL COUNTS

Total and differential white cell counts were made by standard methods, and RA cells were detected by means of phase contrast microscopy.

COMPLEMENT

The total haemolytic complement (CH_{50}) was assayed by incubating serum or synovial fluid with sheep red cells coated with rabbit haemolysin. Lysis was detected with a spectrophotometer and results expressed in terms of the titre of serum or joint fluid which gave 50% lysis.

RHEUMATOID FACTOR

Serum rheumatoid factor was detected by means of a latex agglutination slide screening test using the method described by Bird and Burr (1962). The sensitized sheep cell agglutination titre was estimated in both serum and synovial fluid by the method of Rose and Ball (Ball, 1950). Both sensitized and unsensitized cell titres were determined in the case of fluid, but hyaluronidase was not used. Results are expressed as the last titre of serum or joint fluid which gave definite agglutination.

Table I *Initial and final diagnosis related to duration of joint symptoms on admission to study*

Duration (m)	Initial diagnosis		Final diagnosis		
			Definite RA	Non RA	Other diagnosis
0-3	Definite	4	3	1	See Table II (Resolved undiagnosed)
	Probable	6	0	6	
	Possible	22	4*	18	
	Monarthritis (unclassified)	3	0	3	
3-6	Definite	7	6	1	PsArth
	Probable	0	0	0	OA
	Possible	1	0	1	
6-12	Definite	0	0	0	Renal transplant, steroid subchondral collapse OA (3 cases) Torn meniscus
	Probable	2	1	1	
	Possible	4	0	4	
12+	Definite	13	11	2	SLE, AS AS SLE Ps Arth Gout OA + pseudogout (2 cases) Toxoplasmosis OA (5 cases) OA + acromegaly Ps Arth SLE Torn meniscus
	Probable	13	6*	7	
	Possible	10	1*	9	
		85	32	53	

* Includes one case of JRA.

Ps Arth=psoriatic arthropathy; SLE=systemic lupus erythematosus; AS=ankylosing spondylitis; OA=osteoarthritis; RA=rheumatoid arthritis.

HISTOPATHOLOGY

The synovium was fixed in 10% buffered formal saline, embedded in paraffin, and sectioned at 4–6 μm . Sections were stained with haematoxylin and eosin, and special stains as required. In about half the patients some synovium was fixed in 70% alcohol, but later this was discontinued.

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The synovium was placed in OCT (Lab Tak) and frozen in freezing isopentane or liquid nitrogen. Sections were then cut at 6 μm and treated with polyvalent antihuman immunoglobulin (Wellcome) and monospecific conjugates (Hoechst) for IgG, IgM, β_{1C} , β_{1A} , and fibrinogen. Sections were viewed by epifluorescence using a Leitz Orthoplan with an HBO 200 Mercury vapour lamp, UG1 or UG5 primary filters and K430 or 460 secondary filters.

Photographs were taken using an Orthomat camera with Tri-x or high speed Ektachrome film.

STATISTICS

Data were arranged in the form of contingency tables and tested by the χ^2 test.

Results

Of the 93 attempted biopsies, insufficient synovium was obtained in 8, leaving 85 patients for analysis. Correlation of initial clinical assessment with final clinical diagnosis is shown in Table I. Classification after follow-up has been grouped according to duration of joint manifestations before biopsy, and related to biopsy findings. Since cases of short duration were

Table II *Diagnosis and results of biopsy performed within 3 months of onset of joint symptoms*

Case no.	Weeks since onset	Diagnosis		Histology	Immuno-fluorescence	
		Initial	Final			
1	3	Possible RA	RA	RA	RA	
2	3	Possible RA	Resolved	Nonspecific	Neg	? Psoriatic arthropathy
3	3	Possible RA	Gout	Nonspecific	Neg	Polyarthritides
4	3	Possible RA	Gonococcal monarthritides	Bacterial infection	Neg	Initial cultures sterile
5	3	Unclass MA	Indefinite	Nonspecific	No result	Resolved
6	4	Unclass MA	Indefinite	Nonspecific	Neg	Resolved
7	4	Possible RA	Toxoplasmosis	Nonspecific	Neg	Polyarthritides
8	4	Possible RA	Rheumatic fever	Normal	Neg	
9	4	Possible RA	Penicillin allergy	Nonspecific	No result	
10	4	Possible RA	Indefinite	No result	Neg	? Rheumatic fever
11	4	Possible RA	Reiter's disease	Nonspecific	Neg	Partial syndrome
12	5	Possible RA	Reiter's disease	Nonspecific	Neg	Partial syndrome
13	5	Possible RA	Reiter's disease	Nonspecific	Neg	Partial syndrome
14	6	Probable RA	Reiter's disease	Nonspecific	No result	
15	6	Probable RA	Indefinite	Normal	No result	Resolved
16	6	Probable RA	Rheumatic fever	Normal	Neg	
17	6	Definite RA	Resolved	RA	Neg	*
18	6	Possible RA	Gout + hepatitis	Nonspecific	Neg	†
19	6	Possible RA	Osteoarthritis	Nonspecific	Neg	
20	6	Possible RA	RA	RA	RA	+ Osteoarthritis
21	7	Unclass MA	Indefinite	Normal	No result	Resolved
22	8	Possible RA	Bacterial infection	Bacterial infection	Neg	
23	7	Definite RA	RA	RA	Neg	
24	8	Possible RA	JRA	Nonspecific	Neg	
25	8	Definite RA	RA	RA	No result	
26	8	Possible RA	AS	RA	No result	
27	8	Possible RA	Indefinite	Nonspecific	Neg	Resolved ? JRA
28	8	Probable RA	Reiter's disease	Nonspecific	Neg	
29	10	Probable RA	Reiter's disease	Nonspecific	Neg	
30	12	Definite RA	RA	RA	RA	
31	12	Probable RA	Rheumatic Fever	Nonspecific	Neg	
32	12	Possible RA	†	Nonspecific	Neg	
33	12	Possible RA	RA	Nonspecific	Neg	
34	12	Possible RA	Hodgkin's disease	Nonspecific	Neg	
35	12	Possible RA	Myxoedema	Normal	Neg	

Neg = no evidence of RA; RA = rheumatoid arthritis; Unclass MA = monarthritides unclassified; AS = ankylosing spondylitis; JRA = juvenile rheumatoid arthritis.

* Serological tests positive for psittacosis/lymphogranuloma venereum.

† Urate crystals in fluid. SCAT 1/1024.

‡ Idiopathic femoral condylar osteonecrosis.

most important to this study, the results of biopsies performed within 3 months of the onset of joint symptoms are shown in relation to initial ARA classification and final diagnosis (Table II). A summary of the remainder of the cases is shown in Table I. On initial assessment 24 of the 85 could be classified as definite RA, 21 as probable RA, 37 as possible RA, and 3 as nonspecific monoarthritis. At the time of final review it was found that 32 of the 85 satisfied the criteria for RA. 29 of these 32 had definite RA, and 3 had juvenile rheumatoid arthritis (JRA). Two of the adult cases with definite RA also had gout. One case with JRA also had acute rheumatic fever. Of the remaining patients, spontaneous resolution precluded a final diagnosis in 6, but in the others it was possible to make a definite diagnosis of some condition other than RA (non-RA) as shown in Tables I and II.

HISTOPATHOLOGY

Fifty-three of the 85 cases showed nonspecific changes. In one case biopsy showed the presence of urate crystals. In two others biopsy strongly suggested septic arthritis when previous fluid cultures had been sterile. Subsequently in one case gonococci were recovered from the fluid. In the remaining 30 cases there were changes suggestive of RA, i.e. (i) increased vascularity, (ii) synovial hyperplasia, (iii) infiltration with mononuclear cells particularly around vessels and occasionally as discrete aggregates, and (iv) fibroblastic and mononuclear cuffing of small vessels (Schumacher and Kitridou, 1972). Fully developed rheumatoid nodules were not seen. Of the 30 cases with histological changes considered to be RA positive, 23 were ultimately classified as RA (Table III). The remaining 7 were classified as ankylosing spondylitis, systemic lupus erythematosus, psoriatic arthropathy, or *Chlamydiae* infection. 9 patients ultimately classified as RA had nonspecific histological changes which were therefore recorded as RA negative in Table III.

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With one exception the biopsies which were classified as negative on histopathological grounds had no significant features when stained by means of direct immunofluorescence. There was some surface staining of fibrin in many cases, but it was concluded that this was of no specific significance. 17 of 74 cases were distinctive in that there was focal anti-IgM staining beneath the synovial lining. This appearance is referred to as RA positive in Table IV. The staining pattern varied (Figure a-c), but the most distinctive feature was the presence of a circular ring of staining which appeared to be related to capillary walls. In some instances anti-IgM stained mononuclear cells, some of which appeared to be mature plasma cells. At times the IgM was associated with β_{1C} - β_{1A} , but

this was relatively infrequent. Although antifibrinogen surface staining showed no qualitative difference between the positive and negative synovia, often there was more fibrin present in positive cases. A minority with intense surface staining also had fibrin around small vessels. There appeared to be a correlation between this perivascular fibrin and the fibroblast proliferation seen in the paraffin sections. It was noteworthy that although IgG was frequently found in the subsynovial tissues, with anti-IgG there were no specific diagnostic features.

The correlation between immunofluorescence and follow-up diagnosis is shown in Table IV. Of the 17 cases with a distinctive IgM staining, 15 had RA, one had ankylosing spondylitis, and the other psoriatic arthropathy. All but one of these 17 cases were considered to be RA positive by histopathology.

COMPLEMENT

Analysis of the results of the CH₅₀ titres in serum and fluid are shown in Table V. The mean ratio of fluid/serum titre was only 24% in the 8 seropositive cases but greater than 50% in seronegative RA and non-RA. In 4 patients with Reiter's syndrome this ratio was 75%.

SYNOVIAL FLUID—WHITE CELL COUNTS (TABLE VI)

Relatively high white cell counts were found in 22 of 26 patients with RA, whereas 35 of 51 non-RA cases had low counts. Although high white cell counts

Table III *Histology*

<i>Histopathology</i>	<i>Final diagnosis</i>		
	<i>RA</i>	<i>Non-RA</i>	<i>Total</i>
RA positive	23	7*	30
RA negative	9	46	55
	32	53	85

* Ankylosing spondylitis 3, systemic lupus erythematosus 2, psoriatic arthropathy 1, psittacosis 1.
 $\chi^2 = 30.1$; $P < 0.001$.

Table IV *Immunofluorescence*

<i>Immunofluorescence</i>	<i>Final diagnosis</i>		
	<i>RA</i>	<i>Non-RA</i>	<i>Total</i>
RA positive	15	2*	17
RA negative	14	43	57
No result	3	8	11
	32	53	85

* Ankylosing spondylitis 1, psoriatic arthropathy 1.
 $\chi^2 = 22.3$; $P < 0.001$.

FIGURE *a* ($\times 640$), *b* and *c* ($\times 540$). Immunofluorescence in synovial biopsies. Circular staining with anti-IgM fluorescein isothiocyanate is shown. Vessel walls and mononuclear cells, including plasma cells, were sometimes difficult to distinguish

Table V Complement in serum and synovial fluid

Final diagnosis	No. of cases	Serum titre*		Fluid titre*		Mean fluid titre* Mean serum titre* (%)
		Range	Mean	Range	Mean	
Seropositive RA Positive Rose and Ball	4	90-130	112	10-40	15	13
Seropositive RA Only Latex positive	4	100-170	145	10-100	50	35
Seronegative RA	12	80-160	122	35-110	67	55
Non-RA Total cases	34	40-200	110	15-200	66	56
OA	10	40-120	86	25-80	50	58
Non-RA Non-OA	24	55-200	120	15-90	67	55
Reiter's	4	120-200	152	70-200	118	75

* Reciprocal of titre (CH_{50}).

Table VI *White cell counts in synovial fluid*

White cell count	Final diagnosis		Total
	RA	Non-RA	
$>5.0 \times 10^9/l$	22	16	38
$<5.0 \times 10^9/l$	4	35	39
Not done	6	2	8
	32	53	85

$\chi^2 = 19.5$; $P < 0.001$.

Conversion: SI to traditional units—Leucocytes: $1.0 \times 10^9/l \approx 1000/mm^3$.

were of little diagnostic value, low counts were distinctly unusual in RA. Differential white cell counts are shown in Table VII. High percentages of neutrophils were found in many conditions other than RA.

RA CELLS

40 synovial fluids were examined for the presence of RA cells but in only 2 of 19 cases of RA was the test positive. RA cells were found in one case with SLE. Because of the low sensitivity (in our hands) this test was abandoned.

RHEUMATOID FACTOR

Serum

The Latex screening test was positive in 12 of 32 cases with RA, but it was also positive in 6 of 53 cases without RA. The Rose and Ball test was positive (titre $>1/64$) in 8 of 32 cases with RA (Table VIII). Symptoms in 3 of these 8 had lasted between 2 and 6 months. False positive results were found in 3 cases.

Synovial fluid

The Rose and Ball test on synovial fluid was considered positive when there was a difference of at least 6 doubling dilutions between sensitized and unsensitized titres. Positives were found in only 4 of 32 RA cases and 1 of 42 non-RA (Table IX). If a lower cut-off point (four dilutions) were accepted, 9 of 32 with RA would be positive and so would 4 of 53 with nonrheumatoid disease. In only one instance was the

Table VII *Differential white cell count $>5.0 \times 10^9/l$*

Neutrophil count	Final diagnosis		Total
	RA	Non-RA	
$>75\%$	11	9	20
$60-75\%$	4	3	7
$<60\%$	7	2	9
Not done	1	1	2
	23	15	38

$\chi^2_3 = 1.4$; not significant.

Table VIII *Serum sheep cell agglutination test*

SCAT (titre)	Final diagnosis		Total
	RA	Non-RA	
1/128 or higher	8	1	9
1/64	—	2	2
$<1/64$	24	45	69
No result	—	4	4
	32	52	84

$\chi^2 = 10.1$; $\geq \alpha^2_4 v. < \alpha^2_4$; $P < 0.001$.

Table IX *Synovial fluid sheep cell agglutination test*

Difference between sensitized and unsensitized titres	Final diagnosis		Total
	RA	Non-RA	
6 + dilutions	4	1	5
5 + dilutions	3	3	6
4 + dilutions	2	—	2
<4 + dilutions	23	36	59
Unknown	—	13	13
	32	53	85

$\chi^2 = 3.94$; $>4 v. <4$; $P = 0.05$.

titre of rheumatoid factor in synovial fluid significantly positive (4/512) in the presence of a negative serum.

Discussion

Although past work has suggested that examination of synovia by needle biopsy and tests on joint fluid provide little significant information, the purpose of this study was to discover laboratory means of earlier diagnosis of RA than is at present possible clinically. In this study the findings from synovial fluid examination essentially confirm past reports. The main information from white cell counts appears to be the identification of inflammatory synovitis rather than RA in particular.

Joint fluid rheumatoid factor titres generally reflected those in the serum. A positive fluid in the presence of a negative serum occurred only once, and this patient had obvious erosive disease. Overall, the fluid rheumatoid factor determination did not add diagnostically to the serum estimation.

The Latex screening test correlated poorly with the presence of RA. Performed according to the method described by Bird and Burr (1962), it does not entail coating the Latex particles with gammaglobulin. Although no comparison was made, it appears to be less reliable than the usual latex fixation procedures and is no longer used.

Estimation of complement activity (CH_{50}) in synovial fluid from RA gave distinctive results in only

four instances, when a large (eightfold or more) reduction relative to the serum was found. It was noted that this reduction occurred only in seropositive patients, so that diagnostically the procedure added nothing to information from the sheep cell agglutination test. Synovial fluids from patients with Reiter's syndrome showed a high level of CH₅₀ activity. The mean ratio of fluid to serum CH₅₀ was 75%, which was distinctly greater than in other conditions, and consistent with previous findings (Pekin, Malinin, and Zvaifler, 1967).

Examination of fluid for RA cells was unrewarding, and the previous reported correlation between low CH₅₀ levels in synovial fluid and large numbers of RA cells (Peltier, Delbarre, and Krassinine, 1967) was not found.

The results of this study suggest that needle biopsy of the synovial membrane is diagnostically helpful even at a relatively early stage. Although symptoms lasted only 3 weeks in one patient (Case 1, Table II) of uncertain clinical diagnosis at presentation, RA was strongly suggested by the biopsy results. A second patient (Case 20) had a 5-week history of joint symptoms clinically attributable to obvious osteoarthritis. Biopsy showed histological and immunofluorescent features enabling the identification of superimposed RA.

Needle biopsy appears to be more valuable in terms of diagnostic sensitivity than specificity. Although neither histopathology nor immunofluorescence showed features pathognomonic of RA, the former does allow categorization as mononuclear synovitis, which in this study implied a differential diagnosis of RA, systemic lupus erythematosus, ankylosing spondylitis, or psoriatic arthropathy. If immunofluorescent techniques are applied, some of the biopsies histologically indicative only of mononuclear syno-

vitis can be reclassified as RA with reasonable confidence. Immunofluorescent features considered positive for RA were found inappropriately only in single cases of ankylosing spondylitis and psoriatic arthropathy.

Deciding whether RA has developed in addition to pre-existing osteoarthritis is a common problem, and one which was present in 12 cases of longest duration, as well as in the one already cited (Case 20). 11 of these 12 were confirmed as noninflammatory while the biopsy from the remaining cases produced histological and immunofluorescent features strongly suggesting the unexpected presence of RA.

As shown in one case of gout and 2 of septic arthritis in this series, it is possible for histopathology to show pathognomonic features of conditions other than RA even though clinical features conforming to ARA criteria permit a diagnosis of RA (Schumacher and Kulka, 1972).

It is concluded that synovial needle biopsy may strongly suggest a firm diagnosis of RA before diagnosis is possible clinically, or may indicate the presence of RA in addition to some other condition. It may also lead to recognition of conditions other than RA. Immunofluorescent techniques appear to increase diagnostic specificity, but wider use to test this conclusion further is suggested by the present data.

The needle procedure in this study was not accompanied by any complications. It is simple to do and should be performed more frequently in early doubtful cases of suspected joint inflammation.

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References

- BALL, J. (1950) *Lancet*, **2**, 520 (Serum factor in rheumatoid arthritis agglutinating sensitised sheep red cells)
- BIRD, R., AND BURR, R. G. (1962) *J. clin. Path.*, **15**, 472 (A simple latex slide test for rheumatoid arthritis using the gamma globulin in the patient's serum)
- CAUGHEY, D. E., AND HIGHTON, T. C. (1965) *N.Z. med. J.*, **64**, 444 (Needle biopsy of the synovial membrane)
- HURD, E. R., KINSELLA, T. D., AND ZIFF, M. (1971) *J. exp. Med.*, **134**, 296S (Immunohistologic studies of synoviocytes and synovial exudate cells)
- KINSELLA, T. D., BAUM, J., AND ZIFF, M. (1969) *Clin. exp. Immunol.*, **4**, 265 (Immunofluorescent demonstration of an IgG- β_2 C complex in synovial lining cells of rheumatoid synovial membrane)
- MIKKELSEN, W. M., DUFF, I. F., CASTOR, C. W., ZEVELY, H. A., AND FRENCH, A. J. (1958) *Arch. intern. Med.*, **102**, 977 (The diagnostic value of punch biopsy of the knee synovium)
- PARKER, R. H., AND PEARSON, C. M. (1963) *Arthr. and Rheum.*, **6**, 172 (A simplified synovial biopsy needle)
- PEKIN, T. J., MALININ, T. I., AND ZVAIFLER, N. J. (1967) *Ann. intern. Med.*, **66**, 677 (Unusual synovial fluid findings in Reiter's syndrome)
- PELTIER, A. P., DELBARRE, F., AND KRASSININE, G. (1967) *Ann. rheum. Dis.*, **26**, 528 (Haemolytic complement level and ragocytes (RA cell) concentration in synovial fluid)
- POLLEY, H. F., AND BICKEL, W. H., (1951) *Ann. rheum. Dis.*, **10**, 277 (Punch biopsy of synovial membrane)
- RODNAN, G. P., YUNIS, E. J., AND TOTTEN, R. S. (1960) *Ann. intern. Med.*, **53**, 319 (Experience with punch biopsy of synovium in the study of joint disease)
- ROPES, M. W., BENNETT, G. A., COBB, S., JACOX, R., AND JESSAR, R. A. (1958) *Bull. rheum. Dis.*, **9**, 175 (1958 revision of diagnostic criteria for rheumatoid arthritis)

- SCHUMACHER, H. R., AND KITRIDOU, R. C. (1972) *Arthr. and Rheum.*, **15**, 465 (Synovitis of recent onset. A clinicopathologic study during the first month of disease)
- , AND KULKA, J. P. (1972) *New Engl. J. Med.*, **286**, 416 (Needle biopsy of the synovial membrane—experience with the Parker-Pearson technic)
- TURSI, A., TRIZIO, D., AND BONOMO, L. (1970) *Clin. exp. Immunol.*, **6**, 767 (An immunofluorescence mixed staining technique for the detection of IgG, rheumatoid factor and IgG- β_{1C} complexes in tissues)
- WILKINSON, M., AND JONES, B. S. (1963) *Ann. rheum. Dis.*, **22**, 100 (Evaluation of needle biopsy of synovial membrane)