FII HAEMAGGLUTINATION TEST FOR SERUM ANTIGAMMAGLOBULIN FACTORS IN ARTHRITIDES SERO-POSITIVE AND SERO-NEGATIVE BY OTHER TESTS

BY

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The serological tests for rheumatoid serum factors fall into two categories, in which rabbit antibody and human gamma globulin respectively provide the reactant. Clinically, the chief use of these tests for serum antiglobulin factors is to assist diagnosis of rheumatoid arthritis, and serum titres above given levels are commonly regarded as "positive". In most tests the "positive" level has been selected to include as many rheumatoid arthritic subjects as possible, but at the same time to exclude most non-rheumatoid subjects. Nevertheless it is well-known that a proportion of cases of undoubted rheumatoid arthritis assessed in this way are "sero-negative"; and in juvenile chronic polyarthritis (Still's disease) especially this sero-negative proportion is large. The question therefore arises whether quantitative measurement by a sensitive method might reveal the presence of rheumatoid antiglobulin serum factors in "sero-negative" patients. The FII haemagglutination test originally devised by Heller, Jacobson, Kolodny, and Kammerer (1954) appears to provide such a method. This test detects antiglobulin antibody in patients' sera by haemagglutination of tannic acid treated sheep red cells coated with Cohn Fraction II gamma globulin prepared from human serum. Jacobson, Kammerer, Wolf, Epstein, and Heller (1956) used this method to test sera from a large group of patients with rheumatoid arthritis and other arthritides, and from non-rheumatic patients, and compared results with those obtained by a haemagglutination test using sheep cells sensitized with rabbit anti-sheep antibody. In the FII test they accepted as positive titres of 1:28 or more, and in the sensitized sheep cell test 4-fold enhancement or more of the agglutination titre when sheep serum diluent was used instead of saline. They found good agreement between the two tests when positivity was defined in this way. Others have reported that the FII test is considerably

more sensitive than the latex or differential agglutination test for rheumatoid factor (Peltier and Christian, 1959; Oreskes and Siltzbach, 1968; Oreskes, Rosenblatt, Spiera, and Meadows, 1968).

This paper presents results obtained with the FII test on sera from healthy subjects and from patients with arthritis or other diseases; the sera were also tested by two other methods—the differential agglutination test and latex particle agglutination, representing rabbit and human reactants respectively. In particular, the investigation sought to establish whether the FII test could detect serum antigammaglobulin factors in conditions usually regarded as sero-negative.

Material and Methods

Sources of Sera Tested

Sera from all patients in the wards of this Unit or attending its clinics for whom a differential agglutination test or latex tests was requested between May, 1966, and August, 1967, were also tested by the FII method. This report deals with results obtained on single serum samples received from each patient during this period of time. Control sera were obtained as described. The sex distribution varied somewhat between the different groups of cases distinguished, but this could not be related to the serological findings.

Methods

Differential Agglutination Test (DAT).—This was performed as described by Bywaters and Scott (1960).

The latex fixation test was performed by the single tube method, based on Singer and Plotz (1956) as described by Bywaters and Scott (1960).

FII Haemagglutination Test

(a) *Preparation of FII Coated Tanned Sheep Cells.*—Sheep blood was taken from freshly slaughtered sheep into acid-citrate dextrose solution and kept at 4°C. for 1 week. Sheep red cells were washed four times in phosphate buffered saline (0.15 M Na₂HPO₄ 4720 ml., 0.15 M KH₂PO₄ 280 ml., 0.15 M NaCl 5000 ml.,

pH 8.0). The packed washed cells were diluted with 2 vols buffer.

A solution in saline of tannic acid (Mallinckrodt) containing 1 part in 40,000 was prepared from a 1 per cent. solution kept at -20°C . The cell suspension and the tannic acid solution were both adjusted to pH 8.0.

To the 1:40,000 tannic acid solution an equal volume (usually 24 ml.) of cell suspension was added drop by drop, with constant stirring, and the mixture was incubated for 15 min. at 37°C . It was then centrifuged at 4°C . at 1,000 r.p.m., and the deposit of tanned cells re-suspended and washed in an equal volume of buffer, centrifuged again, and re-suspended in twice the packed cell volume of buffer.

From 1 per cent. solution in buffer of FII (Squibb, Batch 2001) kept at -20°C ., a 1:20 dilution was prepared in buffer.

One vol. (10 ml.) tanned cell suspension was added to 2 vols 1:20 FII solution, and incubated with occasional stirring for 35 min. After cooling to 4°C . the coated cells were centrifuged and washed once in buffer, re-suspended in Alsever's solution, and stored, all at the same temperature. Cells tanned but uncoated with FII were also prepared and kept in Alsever's solution.

Each batch of coated tanned cells was tested as follows against positive and negative human serum controls.

(b) Test Procedure

(1) PRELIMINARY ABSORPTION.—The positive and negative control sera (and subsequently sera for test) were diluted 1:25 in buffered saline, *without* previous heat inactivation, and to 2.5 ml. of this dilution in a 4 in. $\times$ $\frac{1}{2}$ in. tube were added 2 ml. of four times washed fresh sheep cells. After overnight refrigeration of 4°C . the tubes were centrifuged and the agglutination tests carried out on the supernatants.

(2) Positive and negative control sera absorbed in this way were diluted in buffered saline containing 1.5 ml. foetal bovine calf serum (Microbiological Associates Ltd., Bethesda) in each litre. For each serum a row of ten doubling dilutions (1:25 to 1:12,800) was prepared in 0.5 ml. volumes. Two additional tubes containing 1:25 and 1:50 dilutions were included for each serum.

(3) Suspensions of coated tanned cells and uncoated tanned cells respectively were prepared by adding 0.25 ml. packed cells to 100 ml. buffered saline containing 0.15 per cent. foetal bovine serum.

The additional two tubes containing 1:25 and 1:50 serum dilutions each received 0.25 ml. of uncoated tanned cell suspension; the row of serum dilutions in tubes 1 to 10 received 0.25 ml. coated tanned cell suspension in each tube. The tubes were well shaken to mix the contents and kept overnight at 4°C . Sedimentation patterns were read the next morning. Agglutinated cells settle as an unbroken carpet covering the bottom of the tube; unagglutinated cells settle as a small button. Tubes receiving uncoated cells show no agglutination when absorption with uncoated sheep cells has been properly carried out.

(4) Positive control sera gave reproducible titres (varying by ± 1 tube) with each new batch of coated tanned cells. The latter were stored at 4°C . in Alsever's solution for not longer than 3 weeks. When haemolysis of any degree occurred on storage the cells were discarded.

Methods of Recording Results

DAT Test.—Sera giving differential titres of 1:16 or more were considered "positive". In the latex test, strong agglutination was scored "++", and weaker but definite agglutination as "+".

FII Test.—Sera were titrated over a range of doubling dilutions from 1:25 to 1:12,800 as described above. To obtain mean titres for comparison of different groups of subjects, the tubes in this dilution range were numbered consecutively from 1 to 10, and the titres of individual sera scored as tube numbers ($0 = < 1:25$; $1 = 1:25$; $2 = 1:50$, . . .). The geometric mean titres and their variances were calculated on this basis of tube numbers.

All three tests were carried out on the same serum sample from each subject, and a total of 1,739 subjects were tested. Each test was read by one of us (C.A.R.) without prior knowledge of the clinical diagnosis or of the results of other tests. After the findings had been recorded, the diagnosis was checked by inspection of the case notes, and when necessary the patient was re-assessed. A total of 47 patients was excluded from the series at this stage, either because they had been allotted double diagnoses (18 cases) or because the diagnosis was still either unknown or incomplete (29 cases). In no case were the serological results taken into account in assessing the correctness of the diagnosis.

Results

Correlation of Results by the Three Tests

(1) A comparison of the latex and FII test results on the 1,692 sera tested, irrespective of diagnosis, is shown in Table I. Of the 1,249 sera negative by the latex test, 979 (78 per cent.) were either negative in the FII test or gave only low titres (1:100 or less). Of the 378 giving "++" latex reactions, 332 (87.6 per cent.) gave high (1:1,600 or more) FII titres also, and only ten (2.6 per cent.) gave negative or low titres. Similarly, a majority of sera with "+" latex reactions gave moderate (1:200 to 1:800) or high FII titres. There is thus reasonably good correlation between results of these two tests.

Table I also shows that 202 (16.2 per cent.) of the latex-negative sera gave moderate FII titres, and 68 (5.4 per cent.) gave FII titres in the high range. It is likely that this reflects in part a greater sensitivity of the FII test, but that some high FII titres occur in the latex negative group suggests

FII HAEMAGGLUTINATION TEST

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TABLE I
COMPARISON OF LATEX AND FII RESULTS IN 1,692 PATIENTS, IRRESPECTIVE OF DIAGNOSIS

Latex	FII titre	<25	25	50	100	200	400	800	1,600	3,200	6,400	12,800 or More	Totals
	Tube Number	0	1	2	3	4	5	6	7	8	9	10	
Positive	++	4	1	1	4	8	10	18	41	58	55	178	378
	+	5	0	1	6	8	3	12	11	10	6	3	65
Negative		663	79	108	129	91	68	43	23	28	8	9	1,249
Totals		672	80	110	139	107	81	73	75	96	69	190	1,692

also a qualitative difference between the behaviour of the reactants in the two tests. Further evidence of this is the occurrence of a small proportion of latex “++” sera in the group giving negative or low titre FII tests.

(2) The DATs of the 1,692 sera are similarly compared with their FII titres in Table II. The incidence of positive DATs was considerably lower than that of positive latex tests, and the correlation between DAT and FII results was correspondingly less good. Thus, although the great majority (92.7 per cent.) of the 220 DAT-positive sera had high FII titres, more than half the high titre FII sera (52 per cent.) had negative DAT tests. This distribution contrasts with that in the latex tests, where only 15.8 per cent. of high titre FII sera were latex-negative. The discrepancy presumably indicates nothing more than that the reactant in the DAT test, rabbit antibody, differs more markedly from the human gamma globulin reactants of the other two tests than these do from each other. Although allowance must be made for qualitative differences between the behaviour of the reactants in the three tests, Tables I and II give some indication in terms of FII titre of the level of serum antiglobulin activity

which may give rise to DAT and latex reactions of a degree acceptable clinically as positive.

Results in Control Groups, Degenerative Joint Disease, and Rheumatoid Arthritis (Table III, overleaf)

(1) CONTROL GROUPS

(a) *Healthy Subjects*.—Sera were obtained from 23 healthy subjects of both sexes forming part of a population survey previously reported (Ansell and Lawrence, 1966). The sera had been kept at -20°C . for 3 years without thawing. Sera were also obtained from 47 medical and administrative hospital staff members of both sexes; these were tested without delay.

(b) *Sick Controls (Adults)*.—This group consisted of 71 patients attending Unit rheumatism clinics for the first time during the period of study, in whom no rheumatoid, degenerative, infectious, or metabolic joint disease, disorders of connective tissue, or systemic conditions associated with arthritis were found.

(c) *Chest Diseases*.—A group of 65 patients admitted to this hospital with disease of the respiratory system also provided sera for control purposes.

TABLE II
COMPARISON OF DAT AND FII RESULTS IN 1,692 PATIENTS, IRRESPECTIVE OF DIAGNOSIS

DAT	FII titre	<25	25	50	100	200	400	800	1,600	3,200	6,400	12,800 or More	Totals
	Tube Number	0	1	2	3	4	5	6	7	8	9	10	
Positive	1: 128								1		3	52	56
	1: 64	1							3	3	6	42	55
	1: 32	1				1	1	1	3	9	2	25	43
	1: 16	3		1	3	3		1	5	16	11	23	66
	1: 8	13		1	5	2	4	2	7	13	8	19	74
Negative	1: 4	45	3	2	10	7	9	4	5	2	6	3	96
	1: 2	64	13	16	20	16	17	13	14	6	11	2	192
	1: 1	545	64	90	101	78	50	52	37	47	22	24	1,110
Totals		672	80	110	139	107	81	73	75	96	69	190	1,692

RESULTS OF DAT, LATEX, AND FII HAEMAGGLUTINATION TESTS IN CONTROL SUBJECTS

Group	No. of Subjects	Age (yrs)		DAT +	
		(Range)	Mean	No.	Per cent.
(1) Controls					
(a) Healthy	70	(17-80)	39	0	0
(b) Sick (adult)	71	(16-82)	40	0	0
(c) Chest diseases	65	(16-76)	53	1	1.5
(2) Degenerative Joint Disease	84	(33-76)	57	1	1.2
(3) Rheumatoid Arthritis					
(a) Classical and Definite	435	(18-84)	53	181	41.1
(b) Probable and Possible	69	(20-79)	50	3	4.3

- (1) The mean titres of groups (a), (b), and (c) do not differ significantly. Their combined mean, 1.5, has been used for comparisons.
 (2) Significantly different from controls ($P = < 0.001$).
 (3) Significantly different from controls ($P = < 0.001$).

TABLE IIIA
TITRES IN THE DAT AND FII TEST COMPARED IN HEALTHY AND SICK CONTROL GROUPS (a), (b), AND (c) COMBINED

FII Titre Tube Number		< 25 0	25 1	50 2	100 3	200 4	400 5	800 6	1,600 7	3,200 8	6,400 9	12,800 or More 10
DAT	1: 16 or More								1			
	1: 8											
	1: 4	6	2		1							
	1: 2	7	2		4	1		2				
	1: 1	110	15	12	11	10	6	6	5	2		3

TABLE IIIB
TITRES IN THE DAT AND FII TEST COMPARED IN CLASSICAL AND DEFINITE RHEUMATOID ARTHRITIS

FII Titre Tube Number		< 25 0	25 1	50 2	100 3	200 4	400 5	800 6	1,600 7	3,200 8	6,400 9	12,800 or More 10
DAT	1: 128 or More								1		3	48
	1: 64	1							2	2	5	35
	1: 32	1						1	1	8	2	20
	1: 16	1				1			2	16	10	21
	1: 8				1	1	4	1	6	8	5	17
	1: 4	4				1	3	2	3		6	3
	1: 2	1	1	1	1	1	5	4	4		5	2
	1: 1	34	1	6	5	10	8	17	20	31	16	16

Table III shows the results of testing in control groups (a), (b), and (c). It will be seen that in the healthy group (a), only the FII test yielded positives, but that most of these were in the low titre range. In the patient control groups (b) and (c), 4 and 11 per cent. respectively were latex-positive, and in both groups this was matched by some increase in the numbers showing FII titres of 200 or more. The cases with positive latex tests all had FII titres. Table IIIA shows that when titres in the DAT and the FII test are compared in groups (a), (b), and (c) combined, the FII titres show a grading from high

to low values that is not apparent by the DAT. Table III also shows mean titres expressed as mean tube number in the FII titration. Statistically the mean titres of the three control groups do not differ significantly, and their combined mean (1.5) has therefore been used for comparison with other groups.

(2) DEGENERATIVE JOINT DISEASE

The sera of 84 patients with uncomplicated degenerative joint disease had FII titres whose mean

I
ND IN PATIENTS WITH DEGENERATIVE JOINT DISEASE AND RHEUMATOID ARTHRITIS

Latex +/+ +		FII Haemagglutination						
No.	Per cent.	Mean Tube Titre	1: 200 or More		1: 25-1: 100		< 1: 25	
			No.	Per cent.	No.	Per cent.	No.	Per cent.
0		1·4 ¹	8	11	24	34	38	55
3	4	1·2	11	16	14	20	46	65
7	11	1·8	17	26	9	14	39	60
1	1·2	1·7	17	20	18	21	49	59
328	75	7·4 ²	377	87	16	4	42	9
21	30	3·8 ³	35	50	11	17	23	33

and range did not differ significantly from that of the control groups. Only one serum was positive in the other two tests.

(3) RHEUMATOID ARTHRITIS

Cases in which a clinical diagnosis of rheumatoid arthritis had been made were classified according to the criteria of the American Rheumatism Association.

(a) *Classical and Definite Rheumatoid Arthritis*

In this group of 435 patients, 42 per cent. had positive DATs, 75 per cent. positive latex tests, and 87 per cent. FII titres of 1: 200 or more. A further 4 per cent. gave low FII titres. The mean FII tube titre of the group as a whole was 7.4, very significantly higher than the control mean (1.5). 42 patients (9 per cent.) gave no FII haemagglutination titres, and of these only three gave a positive latex or DAT test. In Table IIIB the titres in the DAT and FII tests are compared.

(b) *Probable and Possible Rheumatoid Arthritis*

The 69 patients in this group showed a distinctly lower incidence of positive DAT (5 per cent.) and latex tests (30 per cent.) than the previous group, and their mean FII tube titre was also lower (3.8), although still significantly higher than in the controls. Half of these cases had FII titres of 1: 200 or more.

Results in Sero-Negative Arthritis other than Degenerative Joint Disease

Table III shows that low or moderate FII titres were found in some otherwise sero-negative healthy subjects, and also that among patients in the non-rheumatic control groups low and moderate FII titres were noticeably more frequent than positive latex tests. This finding confirmed the suitability

of the FII test as a means of seeking low levels of antiglobulin factors in otherwise sero-negative arthritis. In this category the following groups were tested, and the results are shown in Table IV.

(1) ANKYLOSING SPONDYLITIS (Table IV, overleaf)

The mean FII tube titre of 65 patients with ankylosing spondylitis was 2.1; this was somewhat higher than the mean control titre, but not significantly different from it. 31 per cent. of the cases had titres of 1: 200 or more—again an incidence not significantly higher than in the control groups or in the group with degenerative joint disease. No correlation was found between FII titre and presence or absence of peripheral arthropathy. All patients with ankylosing spondylitis except one were sero-negative by DAT and latex testing.

(2) ARTHRITIS AND GASTROINTESTINAL DISEASE

26 patients with gastrointestinal disorders were tested. Of these, fifteen had ulcerative colitis, eight regional ileitis, and three Whipple's disease. 22 had a peripheral arthritis, complicated by ankylosing spondylitis in two cases, and four had ankylosing spondylitis alone. The mean FII titre of this group as a whole was not significantly different from that of the control groups.

(3) REITER'S SYNDROME

Of twelve patients with Reiter's syndrome, acute peripheral arthritis was present in eleven; the twelfth had recovered from peripheral arthritis but was developing ankylosing spondylitis. The mean FII value of this group was not significantly different from that of the controls.

(4) GOUT

52 patients with primary gout were tested. Their mean FII tube titre did not differ significantly from that of the controls. One case had a positive DAT and three were positive in the latex test.

RESULTS OF DAT, LATEX, AND FII HAEMAGGLUTINATION TESTS IN ANKYLOSING SPONDYLITIS, ARTHRITIS

Disease Group	No. of Subjects	Age (yrs)		DAT +	
		(Range)	Mean	No.	Per cent
(1) Ankylosing Spondylitis	65	(15-65)	38	0	
(2) Arthritis with Gastrointestinal Disease	26	(7-72)	41	0	
(3) Reiter's Disease	12	(20-47)	31	0	
(4) Gout	52	(31-80)	56	1	2

DAT, LATEX, AND FII IN PATIENTS

Group	No. of Subjects	Age (yrs)		DAT +	
		(Range)	Mean	No.	Per cent
Total	42			5	12
(a) "Rheumatoid Arthritis-like" pattern	13	(36-63)	50	3	23
(b) "Psoriatic Arthritis"	29	(14-66)	41	2	7

* Significantly greater than controls groups (a), (b), (c) combined ($P=0.01$).

† Significantly greater than controls groups (a), (b), (c) combined ($P=0.001$).

RESULTS OF DAT, LATEX, AND FII TESTS IN

Group		No. of Subjects	Age (yrs)		DAT +	
			(Range)	Mean	No.	Per cent
Still's Disease	Over 16 years	138	(16-43)	23	12	9
	Active	69			9	13
	Inactive	69	(16-55)	23	3	4
	Under 16 years	119	(2-15)	11	6	5
Controls	Active	84	(4-15)	11	6	7
	Inactive	35			0	
	Under 16 years	(1) 70	(1-15)	8	0	
	Over 16 years	(2) 203	(7-48)	24	1	0.5

(1)=non-rheumatic sick children.

(2)=rheumatic fever follow-up cases.

In summary, the findings in this group show that in four arthritides usually regarded as seronegative, the FII test revealed no higher incidence nor higher levels of antiglobulin factors than might be expected in random samples of hospital patients with other non-arthritic conditions.

Results in Arthritides with Low or Variable Incidence of Sero-Positivity

(1) ARTHRITIS WITH PSORIASIS

A group of 42 patients with arthritis and psoriasis

showed a significantly raised mean FII titre. In the absence of generally accepted criteria for the diagnosis of psoriatic arthritis, this group was subdivided as follows.

(a) Thirteen patients had a symmetrical peripheral arthritis, often with nodules, and clinically indistinguishable from classical rheumatoid arthritis. In these the mean FII titre was 5.5, approaching the value for definite and classical rheumatoid arthritis (7.4).

(b) The remaining 29 patients had features

IV
WITH GASTROINTESTINAL DISEASE, REITER'S DISEASE, AND GOUT

Latex +/+ +		FII Haemagglutination						
		Mean Titre	1: 200 or More		1: 25-1: 100		< 1: 25	
No.	Per cent.		No.	Per cent.	No.	Per cent.	No.	Per cent.
1	1·0	2·1	20	31	12	19	33	50
1	4	1·4	4	15	10	38	12	47
0		1·0	0		5	42	7	58
3	6	1·8	11	21	8	15	33	64

V
WITH PSORIASIS AND ARTHRITIS

Latex +/+ +		FII Haemagglutination						
		Mean Tube Titre	1: 200 or More		1: 25-1: 100		< 1: 25	
No.	Per cent.		No.	Per cent.	No.	Per cent.	No.	Per cent.
9	21	3.0*	14	33	10	24	18	43
7	54	5.5†	8	62	3	23	2	15
2	7	1.9	6	21	7	24	16	55

VI
STILL'S DISEASE AND IN A CONTROL SERIES

Latex +/++		FII Haemagglutination						
		Mean Tube Titre	1: 200 or More		1: 25-1: 100		<1: 25	
No.	Per cent.		No.	Per cent.	No.	Per cent.	No.	Per cent.
27	20	2·8	49	35	31	22	58	43
19	28	3·5	31	45	13	19	25	36
8	12	2·0	18	26	18	26	33	48
15	13	2·1	27	23	34	28	58	49
14	17	2·6	25	30	21	25	38	45
1	3	1·0	2	6	13	37	20	53
3	4	1·3	11	16	18	26	41	58
3	1·5	1·6	37	18	65	32	101	50

consistent with psoriatic arthritis (Reed and Wright, 1966), notably asymmetrical arthritis, tendon sheath synovitis, terminal interphalangeal joint involvement, and a high incidence of psoriatic changes in the nails; sacro-ileitis was often present in addition, and the course of the disease in these patients was frequently mild. In this sub-group the mean FII titre was 1.9, not significantly different from the controls.

The results obtained with all three serological tests are shown for these two subgroups in Table V; taken together, they support the concept that psoriatic arthritis is an entity distinct from rheumatoid arthritis with psoriasis.

(2) JUVENILE CHRONIC POLYARTHRITIS (STILL'S DISEASE)

A total of 257 cases of Still's disease were tested. Of these 119 were under 16 years old, and 138 were first seen and diagnosed in childhood but at the time of testing were over 16 years of age.

For comparison, two control groups of corresponding age distributions were used, in addition to the control groups already described. These were, respectively, a random group of seventy children in hospital with various non-rheumatic conditions, and a group of 203 rheumatic fever subjects undergoing follow-up. Table VI shows the results in Still's disease and in these two additional control series.

In the cases of Still's disease, the incidence of positive DATs was low—9 per cent. in the over-16s and 5 per cent. in the under-16s. In both age groups positive latex tests occurred with about twice the frequency of positive DATs, and FII titres of 1:200 or more were four times as frequent. The highest incidence of the latter (45 per cent.) was seen in cases of Still's disease over 16 years old with clinically active disease, but in both age groups the mean FII tube titre was significantly, though not strikingly, higher than in the respective control groups. In addition to the above, 33 cases of "probable" Still's disease (Ansell and Bywaters, 1962) were tested. Their mean tube titre was 2·1 (see Table VII).

Results in Other Connective Tissue Conditions and Systemic Conditions Associated with Arthritis

There were 138 patients in the study with conditions affecting the connective tissues, or associated with arthritis, but excluded from the categories already mentioned. For each diagnosis in this group of patients numbers were mostly small as Table VII shows; mean FII titres were calculated only for conditions represented by more than ten subjects. Thus in palindromic rheumatism the mean FII tube titre was significantly raised, and 66 per cent. of the patients gave FII titres of 1:200 or more, as compared with 47 per cent. positive by the latex test, and 20 per cent. with positive DATs. In nineteen cases of systemic lupus erythematosus the mean FII tube titre was barely significantly raised, and only a third of the patients had titres of 1:200 or more. In acute rheumatic fever (37 cases) and in erythema nodosum (19 cases), the mean FII tube titres did not differ significantly from the controls.

Discussion

The FII tanned cell haemagglutination test is a sensitive indicator of serum antigammaglobulin activity. In this study it has been used to titrate the latter and the results may be usefully compared with those obtained by the DAT and latex tests which are often interpreted as "positive" or "negative"—terms relating to the likelihood of an accompanying diagnosis of rheumatoid disease.

Not unexpectedly, many sera negative by the DAT and/or latex test proved by the FII method to possess antigammaglobulin activity. On the other hand, few sera positive by one or other of the former gave no agglutination with FII coated tanned cells. Despite the sensitivity of the FII test, however, results show that its use does not materially alter the broad pattern of antiglobulin activity already well established for joint disease by "diagnostic" serological testing. It does, however, define the pattern in more revealing detail, especially as it provides measurements of serum antiglobulin activity in conditions usually considered preponderantly or wholly sero-negative.

The chief findings in this study are summarized in Table VIII. Firstly, serum factors reactive with human gamma globulin were demonstrated by the FII method in 45 per cent. of healthy subjects, and in 37 to 41 per cent. of patients in hospital with various non-rheumatic illnesses. In all these non-rheumatic groups titres were as a rule low, mean titres lying between 1:25 and 1:50. Four other arthritides usually regarded as sero-negative showed very similar levels and incidences of antiglobulin activity by the FII test.

TABLE VII
RESULTS OF DAT, LATEX, AND FII TESTS IN VARIOUS OTHER CONDITIONS NOT MENTIONED IN TABLES I TO IV

Diagnosis	No. of Subjects	Age (yrs)		DAT +		Latex +		FII Haemagglutination				
		(Range)	Mean	No.	Per cent.	No.	Per cent.	Mean Tube Titre	1/200 or More		1/25-1/100	
									No.	Per cent.	No.	Per cent.
Acute rheumatic fever	38	(5-51)	15	0		1	3	2·0	10	26	10	26
Probable Still's disease	33	(5-26)		3	9	4	12	2·1	8	24	12	36
Systemic lupus erythematosus	19	(14-64)	35	1	5	3	16	2·5	6	32	3	16
Erythema nodosum	19	(13-75)	37	0		1	5	2·0	5	26	6	32
Palindromic rheumatism	15	(16-22)	35	3	20	7	47	4·8	10	66	2	13
Systemic sclerosis	7	(7-22)		0		0		—	2		2	
Dermatomyositis	5	(15-68)		0		0		—	0		2	
Polymyositis	3	(41-55)		0		1		—	1		0	
Polyarteritis nodosa	4	(51-71)		1		2		—	2		1	
Behçet's syndrome	4	(16-49)		0		0		—	0		2	
Viral arthritis	7	(4-40)		0		0		—	0		2	
Erythema multiforme	2	(29-41)		0		0		—	0		1	
Henoch-Schönlein purpura	4	(8-21)		0		0		—	3		0	
Panniculitis	3	(16-53)		0		0		—	0		1	
Sarcoid	4	(28-45)		0		0		—	1		0	
Subacute bacterial endocarditis	4	(18-38)		1		2		—	2		0	

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TABLE VIII
SUMMARY OF RESULTS IN RHEUMATOID DISEASE AND CONTROLS

Group	DAT+ Per cent.	Latex + Per cent.	FII Haemagglutination Titre (per cent.)			
			Mean Tube Titre	1: 200 or More	1: 100-1: 25	< 1: 25
Rheumatoid Arthritis	(a) Classical/Definite	41	7.4	87	4	9
	(b) With Psoriasis	23	5.5	62	23	15
	(c) Probable/Possible	5	3.7	50	17	33
Still's Disease	(a) Over 16 years	9	2.8	35	22	43
	(b) Under 16 years	5	2.1	23	28	49
Controls	(a) Healthy	0	1.4	11	34	55
	(b) Sick (adult)	0	1.2	16	21	63
	(c) Chest Disease	1	1.7	20	21	59
	(d) Rheumatic Fever Follow-up Cases	1	1.6	18	32	50
	(e) Sick Children (under 16)	0	1.3	16	26	58

Antiglobulin activity determined by FII haemagglutination tests has also been reported in 11 per cent. of syphilitic sera (Peltier and Christian, 1959), 15 per cent. of post-epidemic Asian influenza (A₂) sera containing viral antibody (Svec and Dingle, 1965), 49 per cent. of acute psychiatric subjects (Oreskes and others, 1968), and 38 per cent. of patients with sarcoidosis (Oreskes and Siltzbach, 1968).

Against this background, all five categories of rheumatoid disease shown in Table VIII proved to have significantly higher incidences and levels of antiglobulin activity; but perhaps the most striking feature they show is a clear grading of sero-positivity. Thus, the mean FII titre of the groups of cases of *classical or definite rheumatoid arthritis* was between 1:1,600 and 1:3,200. Two out of every three of the 26 per cent. of these cases sero-negative by DAT and latex gave FII titres, mostly of 1:200 or more; but 9 per cent. (39 cases) of the classical or definite group were sero-negative by all tests.

In *rheumatoid arthritis with psoriasis*, the mean titre was lower—between 1:400 and 1:800—but, again, two-thirds of the otherwise sero-negative subjects gave haemagglutination titres in the FII test. The core of consistently negative patients in this group was larger, 15 per cent.

In the “*probable and possible*” category, the FII test revealed serum antiglobulin factors in a little over half of the 70 per cent. that by DAT and latex tests were classified as negative. The mean FII tube titre in this category was lower still—between 1:200 and 1:400—and the proportion of cases sero-negative by all three tests had increased to one-third.

Last in order of grading are the two categories of Still's disease, over 16 years and under 16 years. Both had mean titres between 1:50 and 1:100, just significantly higher than in the respective control

groups. The number giving FII tests at or above the 1:200 level was about twice that giving positive latex tests, but in both over-16s and under-16s the proportion of cases sero-negative by all tests approached 50 per cent., a figure not very different from that in the control groups. It was noted nevertheless that the highest incidence of FII titres of 1:200 or more occurred in patients with Still's disease over 16 years old with clinically active disease. A more detailed analysis of serological findings in Still's disease will be reported separately.

These results show that, in rheumatoid disease, the use of the FII test does not in fact result in a sharper serological demarcation from non-rheumatoid conditions. Instead it reveals the graded character of the increased antiglobulin response in the various rheumatoid conditions included in the study. Indeed, throughout the large series of rheumatoid and non-rheumatoid cases and normal subjects reported here, the distribution of antiglobulin activity forms a graded continuum whose existence is clearly demonstrated by the FII titration method, but is much less evident from latex and DAT results as usually interpreted. Ball and Lawrence (1961) have reported, however, that serum activity against heterologous (rabbit) gamma globulin when titrated down to low levels among random population samples in Northern Europe forms a continuous variable. Since the DAT and latex tests may fail to produce a positive result when one exists, and while diagnosis depends upon results of antiglobulin tests together with clinical judgement, the clinician might well consider using the most sensitive test available. Nevertheless, although once again demonstrating a relationship between marked sero-positivity and the classical forms of rheumatoid arthritis, the FII results equally emphasize that about one out of every ten such cases is persistently sero-negative, and that in even fully developed Still's disease sero-negativity remains the rule.

Finally, it may be remarked that these observations make it difficult to accept uncritically any theory that would implicate autoimmunity, exemplified by the production of antigammaglobulin autoantibody (Williams and Kunkel, 1965), as necessary for the development of the rheumatoid diseases considered here, however secure the proposition that it materially influences the course of these diseases once initiated (Duthie, Brown, Knox, and Thompson, 1957).

Summary

Sera positive in the DAT and/or latex tests usually give high or moderately high titres in the FII haemagglutination test, which uses tanned sheep cells coated with human FII gamma globulin as indicator of antiglobulin activity. Less than 1 per cent. of such sera have FII titres of less than 1:25.

In a series of 1,692 sera from both rheumatoid and non-rheumatoid subjects, 21 per cent. of latex negatives, and 52 per cent. of DAT negatives, gave moderate or high FII titres.

Low FII titres (mean 1:25-1:50) were found in 37 to 45 per cent. healthy subjects, and in patients with various non-rheumatoid subjects, including degenerative joint disease, arthritis with gastrointestinal disease, Reiter's disease, and gout; low titres were also the rule in ankylosing spondylitis (mean 1:50-1:100).

In classical or definite rheumatoid arthritis, rheumatoid arthritis with psoriasis, probable and possible rheumatoid arthritis, and Still's disease, mean titres were significantly higher than in non-rheumatoid controls. The respective mean titres of these groups showed a downward gradation from 1:3,200 to 1:100. In classical or definite rheumatoid arthritis, one case in ten was sero-negative by all three tests. The FII results show that the distribution of antiglobulin activity in a mixed population of rheumatoid and non-rheumatoid subjects forms a graded continuum from high levels to zero.

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Le test d'hémagglutination FII employé pour déterminer les facteurs antigammaglobuline du sérum dans les arthritides séro-positives et séro-négatives à d'autres tests

RÉSUMÉ

Les sérums positifs aux tests DAT ou/et latex donnent généralement des titres élevés ou modérément élevés au test d'hémagglutination FII qui emploie des cellules de mouton tannées enduites de gammaglobuline FII humaine comme indicatrices de l'activité de l'antiglobuline. Moins d'un pour cent de ces sérums avait des titres FII de moins de 1:25. Dans une série de 1,692 sérums de sujets rhumatoïdes et non-rhumatoïdes, 21 pour cent négatifs au test latex, et 52 pour cent négatifs au test DAT, donnaient des titres FII modérés ou élevés.

Des titres FII faibles (en moyenne 1:25-1:50) ont été trouvés chez 37 à 45 pour cent des sujets sains, et chez des malades atteints de diverses maladies non-rhumatoïdes comprenant des maladies de dégénérescence des articulations, des arthrites accompagnées de maladies gastro-intestinales, de la maladie de Reiter et de la goutte; des titres faibles étaient aussi la règle dans la spondylite ankylosante (en moyenne 1:50-1:100).

Dans la polyarthrite rhumatoïde classique ou "définie", la polyarthrite rhumatoïde avec le psoriasis, la polyarthrite rhumatoïde "probable" ou "possible" et la maladie de Still, la moyenne des titres était significativement plus élevée que celle des témoins non-rhumatoïdes. La moyenne respective des titres de ces montrait une progression descendante de 1:3,200-1:100. Dans la polyarthrite rhumatoïde "classique" ou bien "définie", un cas sur dix était séro-négatif à chacun des trois tests. Les résultats pour la Fraction II montraient que la distribution de l'activité de l'antiglobuline chez une population mixte de sujets rhumatoïdes et non-rhumatoïdes forme une continuité graduelle partant de taux élevés à zéro.

Prueba de hemaglutinación FII para factores de suero antiglobulinagamma en artritis seropositivos y seronegativos por otras pruebas

SUMARIO

En los ensayos de DAT y/o látex, los sueros positivos generalmente dan títulos altos o moderadamente altos en la prueba de hemaglutinación FII, que utiliza células de oveja curtidas, cubiertas con una capa de globulina gamma FII humana, como indicador de la actividad antiglobulínica. Menos de un uno por ciento de tales sueros tienen títulos FII de menos de 1:25.

En una serie de 1.692 sueros procedentes de sujetos reumatoides y no reumatoides, 21 por ciento de látex negativos y 52 por ciento de DAT negativos dieron títulos moderados o altos de FII.

Títulos FII bajos (media 1:25-1:50) fueron hallados en un 37 a un 45 por ciento de sujetos saludables y en pacientes con varias dolencias no reumatoides, entre ellas enfermedad degenerativa de las articulaciones, poliartitis con enfermedad gastrointestinal, síndrome de Reiter y gota; títulos bajos eran también comunes en la espondilitis anquilopoyética (media 1:50-1:100).

En la poliartitis reumatoide clásica o definida, la poliartitis reumatoide con psoriasis, poliartitis reumatoide probable y posible y el síndrome de Still, los títulos medios eran significativamente más altos que en los testigos no reumatoides. Los títulos medios respectivos de estos grupos mostraban una gradación descendente desde 1:3.200 hasta 1:100. En la poliartitis reumatoide clásica o definida, uno de diez casos resultó seronegativo por los tres ensayos. Los resultados FII muestran que la distribución de la actividad anti globulínica en un grupo mixto de sujetos reumatoides y no reumatoides forma una gradación continua desde los altos niveles hasta cero.