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THE OCCURRENCE OF THE RHEUMATOID FACTOR IN NON - RHEUMATOID DISEASES

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The Occurrence of the Rheumatoid Factor in Non-Rheumatoid Diseases

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INTRODUCTION

The occurrence of an abnormal serum factor in rheumatoid arthritis has been known and actively investigated for the past 20 years[10]. A multitude of serologic tests have been developed in an effort to aid in the diagnosis and study of the disease[7,23]. Rheumatologists have progressively come up with modifications and refinements which have greatly increased both the ease and sensitivity of these tests to demonstrate the presence of the rheumatoid factor[*7,54]. Evaluation of the various tests and determination of the real incidence of the rheumatoid factor in rheumatoid arthritis and related diseases has been attempted innumerable times[9,79]. Although such determinations are made difficult by the frequent inability to make a firm clinical diagnosis, it is probable that the tests now in common usage give a positive result in only 70-80% of patients with rheumatoid arthritis[26]. This situation is made worse by the frequency in which the negative tests are observed in early, mild or atypical cases of rheumatoid arthritis, coupled with the occurrence of positive tests in clinically similar diseases where an accurate diagnostic test is most sorely needed[10]. In spite

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[*] Although use of the term "rheumatoid factor" implies certain properties and qualities which may not be valid, in this thesis the term will be used in its conventional sense. Any factor present in serum which will produce the agglutination of latex particles under appropriate conditions will be accepted as "rheumatoid factor." While this may not be particularly sophisticated it conforms to common usage and the definition of the 1st conference on serological reactions of rheumatoid arthritis (January 1957) which defined "rheumatoid factor" as "the substance in blood of patients with rheumatoid arthritis and some related diseases responsible for agglutination of sensitized sheep erythrocytes and latex particles in the presence of F II . . ."

of these drawbacks, the factor is almost universally thought of as the "rheumatoid" factor and its detection has become a corner stone in the diagnosis of rheumatoid arthritis (and is accepted as one of the 11 major criterion for diagnosis by the American Rheumatism Association[46]). Efforts to increase the sensitivity of the tests through the use of serum fractions and inhibition techniques associated with very rigid selection of clinical cases are able to increase the correlation between the presence of the rheumatoid factor and rheumatoid arthritis to almost 100%[23]. But, in general, increased sensitivity has been paid for by decreased specificity. The present situation is that in spite of refinements these tests are disappointing in the area where the diagnosis is in most serious doubt[10].

The occurrence of the rheumatoid factor in other diseases has been widely reported[55]. Particularly distressing for diagnosis is the high incidence of the rheumatoid factor in the other rheumatoid diseases[54], e.g., disseminated lupus erythematosis where it may approach an incidence of 50%[49]. Positive tests for the rheumatoid factor have been noted in many patients without any evidence of musculo-skeletal disease[16,18]. Among the more common are liver disease[3], Boeck's sarcoidosis[4], pulmonary tuberculosis[5] and lues[6]. Further, rheumatoid factor has been discovered in as much as 4% of a supposedly healthy blood donner group[57] and 33% in relatives of patients with rheumatoid arthritis who themselves seem free of the disease[17].

In spite of these difficulties--both of frequent false positives and false negative reactions--the tests for rheumatoid factor in the diagnosis of rheumatoid arthritis seems to be

very widely used and heavily relied upon.

The very name almost universally employed of "rheumatoid factor" implies the premise "that it is in some way, directly or indirectly, related to rheumatoid arthritis[1]". As yet there has been no evidence that the rheumatoid factor mediates the pathogenesis of the disease analogous to the production of erythroblastosis by Rh antibodies[7]. Repeated transfusions of large amounts of rheumatoid factor into human volunteers produces neither the lesions nor symptoms of rheumatoid arthritis[2]. A second possibility is that the rheumatoid factor is merely a non-specific reflection of synovitis and vasculitis[1]. While this is an attractive hypothesis because it helps explain the widespread occurrence of the rheumatoid factor, real evidence for its being so is also lacking. Some patients with "classical" rheumatoid arthritis and most children with rheumatoid arthritis do not have rheumatoid factor in their blood by the usual tests[10]. Except for patients in remission and patients with diffuse vasculitis and skin ulceration, the titer of rheumatoid factor seems to bear little relationship to the activity of the disease or to variations in the erythrocyte sedimentation rate[7,23]. Finally, there is a third possibility which is particularly attractive at the present. This is that the rheumatoid factor is produced in elevated levels by whatever causes the rheumatoid arthritis but is probably not itself responsible for the pathogenesis of the disease nor is it secondary and dependent upon the lesions[7]. This line of reasoning is analogous to the Wassermann antibody in syphilis. It has the virtue that it frees the rheumatoid factor (and those who study it) from too

intimate an association with rheumatoid arthritis.

The identification of the rheumatoid factor with the 19S macrogammaglobulins[51] and recognition of its similarity in behavior to an antibody has led to the view that rather than reflecting the presence of rheumatoid arthritis per se, the presence of the rheumatoid factor reflects an immunological response of a more general nature[52]. If we were to think of the production of the rheumatoid factor as an immunological response of the body capable of being triggered by the disease rheumatoid arthritis but also by any number of other diseases and states, then perhaps we could feel more comfortable about the widespread occurrence of the rheumatoid factor in non-rheumatoid diseases.

Perhaps, one difficulty in understanding the nature of the occurrence of the rheumatoid factor is that its investigation has in the main been the province of the rheumatologists themselves and they have overworked it in an effort to make something quite specific and diagnostic out of what in reality may turn out to be a very general phenomenon.

Although reports of the presence of the rheumatoid factor in diseases outside of the rheumatoid-collagen-arthritis group have accumulated over the past 20 years[18], few investigators seem to have been impressed by this. Some small groups of patients with non-rheumatoid disease have been studied, but few investigators have seemed interested in seeing just how widespread is the occurrence of the rheumatoid factor in non-rheumatoid illness.

It was felt that valuable information might be derived

by a study of a fairly large group of patients to see who in the population of a hospital possessed the rheumatoid factor and, if possible, try to explain why this might be so. This approach is the reverse of the bulk of the studies on the rheumatoid factor where, in general, groups of patients have been selected on a clinical basis and then tested for the presence of the rheumatoid factor.

One of the things which became apparent early in this study was that the rheumatoid factor is by no means confined to the patients with rheumatoid diseases. Therefore, groups of patients with conditions which seemed to be associated with a high incidence of the rheumatoid factor (chronic and acute liver disease, sarcoidosis, hyperglobulinemia) were collected and studied for the presence of the rheumatoid factor using titering of serum and a preparation of a euglobulin fraction.

The area, then, of this thesis is the occurrence of the rheumatoid in non-rheumatoid diseases. The goals included 1) identification of the incidence of the rheumatoid factor in the hospital population, 2) determination of the levels of rheumatoid factor which may occur with a view to 3) determining whether the titers of rheumatoid factor present might have value in diagnosis, and 4) hopefully, aid in our understanding of the nature of the occurrence of the rheumatoid factor in rheumatoid and non-rheumatoid disease states.

OCCURRENCE OF THE RHEUMATOID FACTOR IN A HOSPITAL POPULATION -- A STUDY OF 500 PATIENTS

The literature on the occurrence of the rheumatoid factor suggests that it is present in a great variety of non-rheumatoid diseases[18,19,20,21,22] and often in an impressive number of cases e.g., liver disease, 32%[3]; Boeck's sarcoidosis, 10%[4]; pulmonary tuberculosis, 18%[5]; lues, 32%[6]; leprosy, 44%[78]. If these figures are at all accurate, they would suggest that the incidence of seropositive tests for the rheumatoid factor in a hospital population might be quite significant.

It has been possible through the use of very sensitive inhibition methods to demonstrate the rheumatoid factor in almost all patients with rheumatoid arthritis whereas it has been felt to occur much less frequently in other arthritides and non-musculo-skeletal diseases[23]. This has supported the belief that the elaboration of a "rheumatoid factor" is in some unknown, yet nevertheless fundamental manner, related to the rheumatoid process[23]. If it is true, however, that as has been suggested, significant numbers of seropositive tests occur in non-rheumatoid diseases, this premise loses most of its force. Miller et al.[19] and Craig et al.[20] concluded that the sensitized sheep cell test was not diagnostic of any specific disease entity. Dresner in his studies[21,22] using the Fraction II latex fixation test concluded, also, that the presence of the rheumatoid factor is not at all specific. Bartfeld[18] using both the sensitized sheep cell test and human Fraction II latex procedures was impressed by the significant numbers of seropositive tests

in non-rheumatoid diseases.

In view of the suggested failure of the various rheumatoid factor tests to be at all specific, it was felt that a study of the incidence of the rheumatoid factor in the serum of a hospital population would be worthwhile and, in particular, to see what diseases might be associated with the "false positive" tests.

Method and Materials

This study is based upon a rapid slide test latex fixation screening for rheumatoid factor of the sera of 598 patients whose clinical condition was unknown at the time but subsequently discovered by follow-up. Since it was desired that the sera represent the ill population of the hospital yet be unknown at the time of screening, all sera sent to the hospital clinical chemistry laboratory for determination of total protein was used. All such sera received during the period June to the middle of August, 1962 was screened. This source of sera was chosen over others such as the blood bank, routine admission serology, etc., since it was felt that the determination while by no means rare would nevertheless tend to exclude patients admitted for minor surgery, delivery, emergency room visits, clinic follow-ups, etc., and tend to give a greater preponderance of medical and surgical patients with severe illnesses. The determination (which cost the patient $10) was ordered and the blood drawn at the discretion of the attending and house staff and represents, then, in general, patients in the wards and clinics of the Grace-New Haven Community Hospital with serious disease.

The blood was allowed to clot at room temperature. The serum was removed and refrigerated. Within 96 hours it was tested for the presence of the rheumatoid factor using the Hyland RA-Test* which is a commercially available macroscopic latex fixation human gamma globulin slide test. The directions supplied by the manufacturer were followed. Both fresh serum and serum which had been "inactivated" by incubating in a water bath at 56°C. for ½ hour were tested. If both the fresh and inactivated serum gave a positive agglutination reaction, the remainder of the serum was frozen for later titering. The method used in the titering was the commercial Difco** Latex Flocculation Test using pooled human plasma fraction (Fraction II) and polystyrene latex particles. The "multiple dilution technique" recommended by the manufacturer was followed.

Armed with the knowledge of the results of the screening test, the patients' clinical histories were abstracted. Particular attention was paid to those persons whose serum contained the rheumatoid factor. A search was made for any evidence of disease in the rheumatoid-collagen-arthritis group before accepting another, even more prominent, diagnosis.

Because some histories were inadequate or unobtainable, it was necessary to screen 598 sera in order to assemble a group of 500 in which both the screening test and adequate abstract of the clinical history were available.

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* Hyland Laboratories, Los Angeles, California.

** Difco Laboratories, Detroit, Michigan.

Results

Figure I gives some of the pertinent characteristics of the group of 500 as well as the results of the screening tests. The average age was 51.2 years with the oldest patient being 99 and the youngest only 3 hours old (an infant who died of erythroblastosis fetalis.) Neither of these extremes in age had the rheumatoid factor. Forty-seven per cent were male patients. Half were ward patients being treated in the New Haven unit. One was a West Haven Veterans Administration Hospital patient (with bronchogenic carcinoma and no rheumatoid factor.) The rest, representing about 10% of the group, were clinic out patients and patients being seen in the staff Diagnostic Clinic.

It was discovered early that the Hyland latex fixation slide test is exquisitely sensitive and gives an inordinately high number of positive reactions when fresh serum was used. Sixty-six of the 500 or 13.2% were positive. This was felt to be due to non-specific, heat-labile agglutinins in the serum (a phenomenon well known in blood banking and hemagglutination tests). Since the rheumatoid factor has been shown to be stable at 56°C.[23,24,25] only the sera which agglutinated the latex particles in both the fresh state and after preliminary inactivation at 56°C. for ½ hour were considered positive for the presence of the rheumatoid factor. In practice no serum which was initially negative became positive following incubation. Only 38 of the 66 sera survived the incubation, so that using the "double positive" criteria only 7.6% of the 500 patients

Figure I

Number of patients studied: 500.

Average age: 51.2 years (Oldest 99 years, youngest 3 hours.)

Sex distribution: 47% males.

Positive latex fixation screening tests:

Fresh serum 66/500 13.2%
56°C. inactivated serum 38/500 7.6%

Cases of rheumatoid arthritis: 9

"Classical" rheumatoid arthritis	5 with 4 positive LF tests.
Suspected rheumatoid arthritis	4 with 1 positive LF test.

Cases of disseminated lupus erythematosis:

6 good cases of SLE with 3 positive LF tests.

Cases of other rheumatoid-collagen-arthritic diseases:

24 cases with 5 positive LF tests.

Patients judged free of rheumatoid-collagen-arthritic disease: 461

Number of positive LF tests: 25/461 5.4%

Number associated with liver disease: 12/25 48%

Number associated with metastatic disease: 7/25 28%

Patients with liver disease: 55

Number with positive LF tests: 12/55 22%

Patients judged free of rheumatoid-collagen-arthritic and liver disease: 406

Number of positive LF tests: 13 3.1%

Characteristics of the hospital population studied and results of the screening tests.

were considered to possess the rheumatoid factor in their blood.

Over-all there were 39 patients who were classed as having a disease in the collagen-rheumatoid-arthritis group. This represents 7.8% of the population studied. Of these 13 or 1/3 had a positive latex fixation screening test. These included 5 cases of classical and 4 cases of suspected rheumatoid arthritis with the first having 4 out of 5 and the latter 1 out of 4 with double positive screening tests. There were 6 clear cases of disseminated lupus erythematosis with 3 having the rheumatoid factor. The remaining 24 represented other rheumatoid-collagen-arthritis entities of which 5 possessed the rheumatoid factor (cf: Fig. II).

Of the 461 patients who were judged to be free of rheumatoid, collagen or arthritic disease, 25 (5.4%) had serum which would agglutinate the latex particles following 56°C. inactivation. As can be seen from Fig. III, these 25 represent a variety of clinical conditions. Certain patterns, however, are apparent. In almost half (12 of the 25) there was documented or at least highly probable liver disease. Chemical evidence of hepatic decompensation and/or biopsy proof of liver disease were present in these cases before the diagnosis of liver disease was made. In the group of 461 patients there were 55 with documented cases of liver disease. Twelve of these 55 (22%) had positive latex fixation screening tests. In eight more agglutinating activity was present in the fresh serum but did not survive incubation at 56°C. Most of the group were cirrhotics (PNC, Laennec's

Figure II

name	screening	titer			diagnosis
		SSC	Latex		
Armstrong	++	1024		44F	RA
Bilodeau	++	1024	1:5120	53M	RA
Lozefski	++			37M	SLE
Ginsberg	++			14F	SLE
Golden	++			69F	RA
Woodward	++	64	1:1280	46F	Dermatomyositis-RA
Johnson	++			55M	Marie-Strumpel Arthritis
Lonsdale	++			64F	Frozen shoulder, osteoarthritis
Bagre	++		1:80	64F	SLE, lupoid hepatitis
Deisture	++		1:1280	81M	Osteoarthritis, ?Felty's syn., ?Sjörgen's syn.
Manzi	++	512	1:160	78F	Osteoarthritis. pneumococcal pneumonia
Seitz	++		1:1280	79F	RA
Johnson	++	1024	1:160	59F	RA

Patients with Positive Latex Screening Test and Probable Rheumatoid-Arthritic Disease

Figure III

name	screening	titer	age	diagnosis
Hopkins	++	1:80	70	PNC
Brinkley	++	Neg	16	Post cardiotomy
Worzina	++		73	Cardio-pul. dis, Tbc.
Bertalovitz	++		60	GI adeno ca. early ?UC
Tarantino	++	1:640	36	Gingovitis, rheumatic heart dis., myocardial strain.
Brady	++	1:80	55	Laennec's & PNC
Frankfurther	++		71	Metastatic ca
Maiorino	++			Metastatic ca
Aymon	++		80	Cardiovascular dis.
O'Neall	++		32	Chronic alcohol, psychiatric dis.
Kelly	++	1:2560	57	Hepatic disease, abnor. LFT's
Warner	++	Neg	48	Primary biliary cirrhosis
Henderson	++	1:160	52	Hepatic disease, undet. etio.
Barnett	++	Neg	64	PNC
Curzi	++	Neg	59	Cirrhosis
Cook	++		57	Metastatic ca
Fredericks	++	1:80	80	CDS with decompensation
Marek	++		66	Metastatic ca
Ely	++		81	Pneumonia
Codianne	++		57	PNC
Roller	++		50	Hepatic disease, abnor. LFT's. old MI, pyelonephritis
Lifshatz	++	Neg	70	Metastatic ca
Carroll	++	1:40	56	Laennec's
Pechnarckk	++	1:160	78	Ca of prostate with invasion of bladder
Stone	++	Neg	33	Post-cardiotomy, ? myocardial abscess, FUO

25 Patients with Positive Latex Fixation Screening Test and without Evidence of Rheumatoid Disease

cirrhosis, one cardiac and one hemosiderotic cirrhotic.) Of 33 cirrhotics, 7 or 21% had the rheumatoid factor and another 4 had agglutinating activity only in the fresh serum.(cf. Fig. IV). There was one patient with hepatitis who also had positive LE preparations and the rheumatoid factor in her blood and she has been classed as a rheumatoid disease (so-called lupoid hepatitis.)

It was observed that the presence of rheumatoid factor in the blood of patients with liver disease was generally, but not invariably, associated with very severe derangement of liver function, active hepato-cellular necrosis and gross hepatic decompensation.

The remaining members of the group without evidence of a rheumatoid-collagen-arthritic disease but with rheumatoid factor and without liver disease (13 of 25) included 7 with metastatic disease. It is interesting that 2 were having post-cardiotomy difficulties (a "Waring blender" hemolytic anemic and a probable myocardial abcess.)

While it was not possible to titer all the patients showing rheumatoid factor, enough information is available to see a clear trend. Fifteen of the 25 non-rheumatoid and 8 of the 13 rheumatoid patients with positive screening for the rheumatoid factor were titered. It can be seen by comparing Figures II and III that the levels of the rheumatoid factor were consistently much greater in the patients with the rheumatoid diseases. In no case was a negative titer found following a positive screening test in the group with rheumatoid disease. However, this was

Figure IV

total	Liver Disease	neg	Positive Latex Fixation Screening Test	
			fresh serum (only)	inactivated
33	Cirrhosis	22	4	7
3	Hepatitis	--	3	-
10	Metastatic	9	-	1
4	Granulomatous	3	1	-
2	Decompensation 2° CDS	-	-	2
1	Lupoid hepatitis	-	-	1
2	Unknown	1	-	1
55		35	8	12

Patients in the Population Studied with Liver Disease

frequently the case in the non-rheumatoid group and further the titer which were present were generally very weak. One patient with liver disease titered to 1:2560 but otherwise the titers were either negative (7 of 15) or at least below 1:160 (13 of 15).

RELATIONSHIP OF HYPERGLOBULINEMIA TO THE RHEUMATOID FACTOR

Bartfeld[16] in his extensive review of the occurrence of seropositive tests for the rheumatoid factor in non-rheumatoid diseases pointed out that the conditions associated with the presence of the rheumatoid factor were also those associated with hypergammaglobulinemia, especially, he thought, primary and secondary macroglobulinemias. Howell et al.[27] and Kunkel et al.[28] also noted this association. Waldenstrom and Winbloc[17] suggested in 1958 that positive sensitized sheep cell test for rheumatoid factor were part of an anamnestic reaction for various immune bodies found in diseases associated with hypergammaglobulinemia. Dresner and Trembly[15] noted in their study that hyperglobulinemic states due to little known causes may (but not invariably) produce the phenomenon of latex agglutination. Examples which they sighted were Waldenstrom's macroglobulinemia, some hyperglobulinemic reticuloses, amyloidosis, and nephrosis. Other authors have observed the same thing about the occurrence of the rheumatoid factor in sarcoidosis and kala-azar[28] and syphilis[28,34].

It was felt that since these observations were made on isolated cases it might be informative to study a series of patients chosen for their hyperglobulinemia to see just how the rheumatoid factor would occur in such a group.

Method and Materials

The total protein determinations done routinely in the Grace-New Haven Community Hospital clinical chemistry laboratory

were observed daily. A value greater than 4.00 grams% of globulin was selected as falling in the hyperglobulinemic range. This laboratory uses the standard salting out-Biuret method of determining total protein and the proportions of albumin and globulin. Fifty-one consecutive sera considered to be hyperglobulinemic were sampled and the presence of latex agglutinating activity determined on the fresh serum using the Hyland R-A screening technique previously described. If agglutination occurred the slide test was repeated on serum inactivated at 56°C. for 30 minutes. If rheumatoid factor activity was present in the inactivated serum the level of activity was determined by titering using the RA Flocculation Test already described. The clinical histories of the patients were abstracted looking particularly for an explanation of the elevated globulin and for any evidence of a rheumatoid disease. In a number of the cases serum electrophoretic patterns were available. The group studied then represents 51 private, ward and clinic patients who had protein determination done at the discretion of the house officers and who had an elevated globulin fraction.

Results

The data are given in Fig. V. The 51 patients exhibited an extremely wide range of diseases with the cause of the hyperglobulinemia not always readily apparent. The average value for the serum globulin fraction in the group was 4.67 grams% with a range of 4.01 to 6.29. No pattern seems obvious. There is no direct relationship between the hyperglobulinemia and the

Figure V

Hyperglobulinemia		Screening	Titer	Diagnosis
1. Anderson	4.98	+-	negx2	?Sarcoidosis
2. Bagre	6.0	++	1/80x2	Lupoid hepatitis
3. Brady	4.7	++	1/80	Laennec's cirrhosis
4. Brevillier	4.2	-		Bronchogenic Ca
5. Brown	4.5	+-	1/2560	Discoid lupus erythem.
6. Copienne	5.1	±-	Negx2	PNC, Laennec's cirrhosis
7. Conley	4.1	-		3° burns c̄ renal shut down
8. Crisci	4.8	+-	1/640	Laennec's cirrhosis
9. Crouse	4.01	--		SLE
10. Crump	6.1	-		Chronic GU infection
11. Curzi	4.6	+±	Neg	Laennec's cirrhosis
12. D'Amico	5.1	±-	Neg	SLE
13. Daren	4.1	-		Acute pyelonephritis, diabetes
14. Deisturo	5.2	++	1/1280	Enteric fever ?Felty's syndrome ?Sjörgen's syndrome, osteoarthritis
15. Dudrick	4.3	-		Cardiac cirrhosis
16. Dubicki	4.1	-		Severe burns
17. Fields	4.78	±-	Neg	Toxic goiter
18. Fountain	4.4			?FUO Normal LFT
19. Garabedian	5.0	-		CDS
20. Grant	4.1	-		?Tbc
21. Greenstein	4.06	-		Subacute necrotizing encephalitis
22. Gross	5.1	-		Bronchogenic Ca
23. Gryga	4.2	+-	Neg	Ca of larynx
24. Henderson	6.0	++	1/160	Boeck's carcoid
25. Hopkins	4.2	++	1/80	PNC
26. Horton	4.7	-		PNC
27. Iler	4.04	-	Neg	Myesthenia grava normal LFT's
28. Kebeian	4.48	+±	1/640	Abdominal angina
29. Kelly	5.6	++	1/2560	Abnormal LFT's with normal SGOT
30. Lewchuk	4.9	±-	1/160	?Tbc
31. Magrey	4.1	-		Laennec's cirrhosis
32. Mangs	4.16	-		Ca of prostate
33. Martin	4.4	-		Constrictive pericarditis
34. Moore	4.4	-		Alcoholism, pancreatitis
35. Moses	4.69	-		Adenopathy, ?Tbc
36. Pavis	4.07	±-	Neg	AGN, Tbc, pneumonia
37. Presutto	4.4	+-	Neg	CDS c̄ hepatic decompensation

Hyperglobulinemia		Screening	Titer	Diagnosis
38. Purcycki	4.4	-		Ca of esophogus
39. Ribes	4.16	+-	Neg	Gastric ulcer
40. Rice	4.4	-		Pick's disease
41. Sullivan	4.3	- -		Ca of cervix and cerrhosis
42. Sweeney	4.3	+-	1/2560	Biliary cirrhosis
43. Tomasi	4.58	-		Carcinomatosis
44. Trapp	4.08	-		Congenital hemolytic spherocytic anemia
45. Travers	5.45	-		Bronchogenic ca
46. Triano	4.99	-	Negx2	Thallosemia major, hemosiderosis
47. Warner	4.1	++	Neg	PNC
48. Wasserman	4.8	±-		ASCVD, CHF, abnormal LFT, pyelo.
49. Whicker	6.29	-		CNA
50. Willis	4.9	-		Chronic pyelonephritis
51. Woodward	6.29	++	1/1280	Dermatomyositis

Latex Fixation Screen Tests in 51 Patients
with Hyperglobulinemia

presence of the rheumatoid factor. The highest value -- 6.29 gm% -- occurred twice. Once in a patient with dermatomyositis and severe rheumatoid symptoms with a high titer of the rheumatoid factor. And once in an elderly Negress with a CVA but no apparent cause for her hyperglobulinemia and no rheumatoid factor in her serum. Over all, 22 of the 51 or a little less than one-half of the patients with hyperglobulinemia showed some agglutinating activity in the fresh serum but this disappeared upon heating in half of these cases leaving only the following 10 with clear rheumatoid factor:

	Globulin	Screening	Titer	Diagnosis
Bagre	6.0	++	1:80	Lupoid hepatitis
Brady	4.7	++	1:80	Laennec's cirrhosis
Curzi	4.6	+±	Neg	Laennec's cirrhosis
Deisturo	5.2	++	1:1280	Possible enteric fever, ?Felty's syn., ?Sjörgen's syn., severe osteoarthritis
Henderson	6.0	++	1:160	Boeck's sarcoid with hepatic infiltration.
Hopkins	4.2	++	1:80	Post-necrotic cirrhosis
Kebeian	4.5	+±	1:640	?abdominal angina with normal LFT's.
Kelly	5.6	++	1:2560	Pneumonitis with markedly abnormal LFT's except normal SGOT.
Warner	4.1	+±	Neg	Post-necrotic cirrhosis.
Woodward	6.3	++	1:1280	Dermatomyositis with Sx of RA.

What stands out is that although hyperglobulinemia is associated with agglutination of the latex particles when fresh serum is used in 50% of the cases this represent not true rheumatoid factor which is heat stable but "non-specific" heat-labile agglutinating activity. When the serum is initially heated to remove this activity what remains is for the most part hyperglobulinemia associated with liver disease.

RHEUMATOID FACTOR IN SARCOIDOSIS

Boeck's sarcoid has been repeatedly[6,7,16,20,28,53,54,55] reported as being associated with the presence of the rheumatoid factor. Kunkel et al.[4] place the incidence at about 10%. Bartfeld[16] reviewed the literature and found that using sensitized sheep cell agglutination tests 11 cases of sarcoidosis were reported with 2 having positive tests for an incidence of 18%. Of 62 cases of sarcoidosis reported where the serum was tested for the rheumatoid factor using F II latex agglutination tests 6 were positive for an incidence of 10%. This incidence is not great considering that 4% of a healthy, normal control group will show the presence of the rheumatoid factor[17]. Nevertheless, the occurrence of the rheumatoid factor in sarcoid is interesting because at least two possible explanations present themselves, viz., the characteristic hyperglobulinemia and often hepatic infiltration by granulomata.

It was felt that the study of a series of patients with clearly established sarcoidosis might be worthwhile. To detect the rheumatoid factor in the sera of these patients, the titering of a euglobulin fraction in addition to that of the whole serum was done in an effort to unmask the presence of the rheumatoid factor.

Method and Materials

Patients were studied who carried the diagnosis of sarcoidosis. Their clinical histories were abstracted and a sample of serum frozen after preliminary screening using the

Hyland R-A slide test. It was soon found that while the diagnosis of sarcoidosis is fairly common (15 cases in 2½ months), proven disease is somewhat harder to come by. The diagnosis was often circumstantial. It was also often complicated by other systemic diseases making study hazardous. Further, sarcoid presented in different ways--some with hilar involvement, some with hepatic infiltration, some with cutaneous lesions, and a few with arthritic symptoms--making comparison difficult. Also, the disease was of variable activity. Selection of suitable cases reduced the group to 8 with biopsy proven sarcoidosis. Six of the eight had proven granuloma in their livers, one had granulomata in a pleural biopsy and the last in a skin biopsy. The patients tended to be young (5 were less than 25) and all but one were females. Half were Negroes. Serum electrophoretic patterns were available on all but one. Screening with the latex agglutination slide test and titering of the serum and euglobulin fraction were done as previously described. The procedure used for precipitation of the euglobulin fraction is described in the Appendix. Euglobulin precipitation was not possible in two patients.

Results

The data are given in Fig. VI. Of this small but carefully selected group of 8 patients with clinical sarcoidosis proven by biopsy, only one had a titerable amount of rheumatoid factor in the serum. Titering of the euglobulin fraction was negative in all six tested including the patient (Henderson) with the serum titer of 1:160. Four did have positive latex

fixation screening tests using fresh serum but this was associated in each case with a globulin fraction greater than 4.0 grams%. Only in two of the eight did the agglutination persist in the 56°C. inactivated serum (Henderson and Davis). One had a titer of 1:160 in her serum and the other was negative on titering. In two cases where the disease was active enough to require hospitalization and corticoid therapy (Davis and Anderson), the rheumatoid factor was not present in titerable amounts.

Figure VI

Name		Dx	A/G	γ-globulin	Screening	Titer Serum	Euglobulin	LFT's
Upjohn	20W	Pulmonary sarcoidosis	2.9/3.2	normal	Neg	--	Neg	normal
Schmickel	22W	Generalized sarcoidosis c̄ hepatic granuloma	3.9/2.5	normal	Neg	--	Neg	normal
Henderson	18N	Generalized sarcoidosis c̄ hepatic granuloma	2.9/4.2	1.97	++	1:160	Neg	normal
Foth	52W	Generalized sarcoidosis c̄ hepatic granuloma "burnt out stage"	3.8/3.1	normal	Neg	--	Neg	grossly abnormal
Murray	60N	Generalized sarcoidosis c̄ hepatic granuloma uveitis	3.2/4.6	--	+-	--	--	Alk PO_4ase 48
Anderson	17N	Generalized sarcoidosis c̄ hepatomegaly uveitis	2.8/5.0	2.31	+-	Neg	Neg	borderline normal
Davis	24N	Generalized sarcoidosis c̄ hepatic granuloma	2.3/4.3	1.92	++	Neg	Neg	normal except BSP 9%
Lavieri	31W	Generalized sarcoidosis c̄ hepatic granuloma	3.9/2.6	0.87	Neg	--	--	Alk PO_4ase 33 SGOT 65 BSP 17%

Patients with Sarcoidosis

LIVER DISEASE AND THE RHEUMATOID FACTOR

Work by many authors[3,7,13,15,18,27] has shown an increased incidence of the rheumatoid factor in liver disease. Dresner and Trembly[22] compiled data from six papers reporting 28 cases of liver disease tested as non-rheumatoid controls and showed an incidence of about 68% positive latex fixation or hemagglutination tests. They concluded that the agglutination phenomenon in hepatocellular disease is associated with the constant presence of hypergammaglobulinemia. These authors went on to study the electrophoretic and ultracentrifugation properties of sera from four patients with cirrhosis and showed the agglutinating activity to be a gamma globulin with a sedimentation constant of 19 or 22S similar to that of the rheumatoid factor. Howel et al.[3] also studied the 19S gamma globulins produced in liver disease and were unable to find any difference between them and the rheumatoid factor. Bartfeld[16] reviewed the literature and collected the following information about the occurrence of rheumatoid factor in liver disease:

	No. of Papers	No. of Patients	No. Positive	% Positive
Infectious Hepatitis	7	59	15	25%
Liver disease	8	191	34	18%

His overall figure of about 20% corresponds well with the value of 22% found in the 55 patients with liver disease studied as part of the 500 already reported in this thesis.

Since half the non-rheumatoid patients in the group of 500 studied who had a positive slide test for the rheumatoid

factor had liver disease and most of the group with elevated globulin with a positive test also had liver disease, it was felt that useful information might be obtained by collecting a series of patients with known liver disease. The availability of Dr. Klatskin's private diagnostic clinic and hospital consulting service made the assembling of thoroughly worked-up patients with proven liver disease feasible. The patients were screened in the usual manner. Titering of their serum and euglobulin fraction was done in an effort to get a clear picture of the occurrence of the rheumatoid factor in liver disease.

Method and Materials

Thirty patients with severe liver disease were studied. In 24 the clinical impression was confirmed with a tissue diagnosis. Fig. VII gives a breakdown of the types of disease in the group. Twenty-four had cirrhosis. Five had a viral hepatitis (including the hepatitis of infectious mononucleosis as a viral disease.) One had subacute hepatic necrosis. One had granulomatous infiltration secondary to infestation with Schistosoma mansoni. Serum from these patients was frozen after preliminary screening for the rheumatoid factor using the Hyland R-A macroscopic slide test on both fresh and 56°C. inactivated serum. At a later date the serum and euglobulin fraction were titered for rheumatoid factor activity using the latex-F II flocculation procedure previously described. Electrophoretic patterns were available on most of the group.

RESULTS

The results are presented in Fig. VIII. Of the 30 patients, 9 (33%) had positive screening tests in both the fresh and inactivated serum. This was most common in the cirrhotics (8 of 24) who made up the bulk (24 of 30) of the group. One of the four with acute viral hepatitis had a positive screening test. Positive titers on the fresh serum were seen only in the cirrhotics (4 of 24). Positive titers using the euglobulin fraction were present in 4 patients--2 cirrhotics and 2 with viral hepatitis. Correlation between the three tests for the rheumatoid factor was poor. Several patients showing titers with the euglobulin fraction had negative titers using the serum and negative screening test. The gamma globulin content of the sera was generally elevated but unassociated with the presence or absence of the rheumatoid factor. Prior to inactivation at 56°C. 19 of the patients had agglutinating activity in their serum (63%) but only in 9 did this survive the heating.

Figure VII

		Positive inactivated screen	Titer	
			serum	euglobulin
Cirrhosis				
Laennec's	10	111	11	0
PNC	9	1111	11	1
Biliary	3	1	0	1
Cardiac	1	0	0	0
Hemosiderotic	1			
Sub-acute hepatic necrosis	1	0	0	1
Viral Hepatitis				
Infectious	1	0	0	0
Infectious Mononucleosis	2	1	0	1
Serum	1	0	0	0
Schistosoma granulomata	1	0	0	0
	30	9	4	4

Latex Fixation in Various Types of Liver Disease.

Figure VIII

			Titer		
		screening	fresh	euglobulin	γ-glob
Martinez	Laennec's	+-	Neg	Neg	3.42
Hopkins	PNC	++	1/80	1/80	1.98
Bussing	Biliary cirrhosis	+-	Neg	Neg	2.19
McLaughlin	Laennec's	--		Neg	
Simmons	PNC	±-		Neg	1.91
Triano	Hemosiderotic cirrhosis	+-	Neg	Neg	
Lyons	PNC (lupoid hepatitis)	--		Neg	1.85
Bernstein	Sub-acute hepatic necrosis	--		1/40	
Barnett	PNC	++	Neg	Neg	
Levine	Serum hepatitis	±-	Neg	Neg	1.04
Greene	Biliary cirrhosis	+±	Neg	1/80	2.21
Rice	Viral hepatitis (IM)	+±	Neg	1/80	
Dauria	Viral hepatitis	--		Neg	1.17
Horton	PNC	-		Neg	
Brady	Laennec's	++	1/80	Neg	2.15
Sekas	Viral hepatitis (IM)	+-	Neg	Neg	
Codianne	PNC	±-	Neg	Neg	2.55
Mughir	PNC	+±	1/160	Neg	1.53
Dudrick	Cardiac cirrhosis	--		Neg	
Strand	Laennec's	-		Neg	
Warner	PNC	+±	Neg	Neg	
Fisher	Laennec's	+±	Neg	Neg	2.85
Magery	Laennec's	--		Neg	1.91
Carmon	Laennec's	+-	Neg	Neg	2.79
Hernandez	Schistosoma granuloma	-		Neg	1.15
Medford	Laennec's	+-	Neg	Neg	
Carroll	Laennec's	++	1/40	Neg	
Presutto	Biliary cirrhosis	+-	Neg	Neg	1.56
Nettleton	Laennec's	-		Neg	1.59
Kupfer	PNC	-		Neg	

30 Patients with Documented Liver Disease

DISCUSSION

The study of 500 ill patients revealed that the incidence of the rheumatoid factor when 56°C. inactivated serum is screened is 5.4% in patients without evident rheumatoid disease. This is a much lower figure than was predicted after review of the literature[10]. Healthy people serving as blood donors are reported to have only a slightly lower (4%) incidence[57]. However, when the group is analyzed it is found that the positive latex agglutination tests are not scattered at random, rather half are associated with liver disease and another one-forth have terminal metastatic disease. When both patients with probable rheumatoid disease and/or liver disease are eliminated from the group of 500 the incidence of positive tests falls to 3.1%; about what one might expect of a healthy population[79].

The incidence of positive tests among those with liver disease was 22%--a five-fold increase over Waller et al.'s[57] group of blood donors. This value agrees tolerably well with the incidence of 33% in the 30 patients collected specifically for their liver disease. The incidence in patients selected for their hyperglobulinemia again pointed toward liver disease as the source of the rheumatoid factor in non-rheumatoid disease. deForest[44] obtained 313 sera from the serological laboratory of the Grace-New Haven Hospital representing patients with diverse disease. Twenty or 6.4% gave positive tests using the F II latex agglutination test. Over half of these patients giving seropositive tests were diagnosed as having some hepatic disorder--mostly cirrhosis and hepatitis. The agreement between these

findings and the group of 500 from the same hospital reported in this thesis is quite close.

It seems clear on the basis of the data presented in this thesis that the over-all incidence of the rheumatoid factor in non-rheumatoid disease is not great, say 5%, when heated serum is used for screening, but also that the incidence is considerable in liver disease. Dresner and Trembly[21] compiled data from 6 papers concerning 28 cases of liver disease used as non-rheumatoid controls and found a mean incidence of 68% positive latex agglutination or hemagglutination tests. Bartfeld[18] attempted the same thing and found an over-all incidence of 20% in 250 patients, closer to the figure observed in this thesis. The observation made by Ziff[9] after noting an incidence of 16% in patients with infectious hepatitis; " . . . the possibility exists that in the presence of liver disease abnormal globulins are synthesized which have agglutinating activity;" seems amply demonstrated.

The suggestion, often made[15,16,17,27,28], that the increased incidence of rheumatoid factor activity in liver disease and the other non-rheumatoid diseases is associated primarily with the presence of hypergammaglobulinemia is not wholly supported here. It seems rather that in hyperglobulinemia it is liver disease that produces both the elevated globulin and rheumatoid factor. Certainly from the 51 patients studied, no clear association between increased globulin and agglutinating activity was apparent independent of liver disease when non-specific ag-

glutinating activity was removed by heating. That 50% of hyperglobulinemic sera contains heat-labile agglutinating activity is only significant as a possible source of confusion with the heat-stable rheumatoid factor. In the cases of sarcoidosis studied, where hypergammaglobulinemia was common as shown by the electrophoretic patterns (cf. Fig VI), elevated gamma globulin was present in both cases which gave a positive screening test. In the 30 patients with liver disease, in every case (5) where the screening test was positive and the gamma globulin fraction known it was seen to be elevated. Hence, the contention of Dresner and Trembly[21] that "the feature common to all the non-rheumatic states associated with latex fixation is the presence of a fast-moving macroglobulin . . ." is at least not contradicted by the data available. The other side of the coin, however, is that the electrophoretic patterns revealed much more hypergammaglobulinemia than positive latex fixation tests.

Why in liver disease there should be small but detectable amounts of rheumatoid factor is a mystery. Unlike rheumatoid disease, the occurrence may be a transient phenomenon. Of 19 cirrhotic patients studied by Dresner and Trembly[21] who had positive latex agglutination tests, 53% reverted to negative with clinical remission of the disease. These authors go so far as to suggest the presence of rheumatoid factor may be a subtle test for active hepatocellular damage and reversion from positive to negative as a good prognostic sign. The results in this thesis do not suggest such a close correspondance between

liver disease and rheumatoid factor as Dresner and Trembly envision. Positive latex screening tests were generally observed to be associated with severe derangement of liver function with active hepato-cellular necrosis but in some cases with gross hepatic damage and decompensation the rheumatoid factor was not detected.

All efforts aimed at demonstrating physical, chemical or immunological differences between the macroglobulins responsible for latex agglutination in rheumatoid and non-rheumatoid diseases have been unsuccessful[21,23,27,28]. The methods, however, are still relatively crude and a final answer is yet to come. There is reason to hope that the new technique of immunoelectrophoresis will be able to differentiate specific components in the agglutinating activity.

The question, Why should a phenomenon strongly associated with rheumatoid arthritis occur in liver disease? is unanswerable at the present time but there are certain clues. In liver disease major but little understood immunological changes occur[61]. The production of gamma globulins, as it were, seems to go out of control and there is a generalized amnestic immunological response. Patients with liver disease produce besides the rheumatoid factor, anti-nuclear factor[61], Wassermann antibody[61], organ specific-compliment fixing antibodies[62], and antibodies to liver cell products themselves[63]. Havens[64] demonstrated the ability of patients with cirrhosis to produce greatly augmented levels of specific antibodies following injection of tetanus or diptheria toxin. Paronetto et al.[63] using a fluorescent-antibody

technique were able to demonstrate the local formation within hepatic reticulo-endothelial cells of gamma globulin and hence the source of the macroglobulins seems to be the mononuclear inflammatory infiltrate seen in the diseased liver[65]. This association of a heavy plasmacytic reaction and elevation in certain gamma globulins has prompted the term "plasma cell hepatitis[66]".

This evidence suggests that the occurrence of the rheumatoid factor in liver diseases in only part of a totally non-specific production of macroglobulins by a diseased R-E system. Simply stated, the immunological apparatus of the mononuclear cells which are greatly multiplied in the liver has gone haywire and is producing small amounts of many of the globulins for which the plasma cells have templates[*].

A theory to explain the occurrence of the rheumatoid factor in non-rheumatoid diseases based upon what appears to be occurring in liver disease can be constructed. Lupoid hepatitis has been extensively studied[71] and may serve as a model. This is a chronic hepatocellular disease, usually in non-alcoholic subjects, with high gamma globulin and dense infiltration of the liver with lymphoid cells (plasma cell hepatitis.) Dr. Klatskin[60] is of the opinion that this entity goes on ultimately to post-necrotic cirrhosis. The present concept of the pathogenesis is that there is colonization of the liver (perhaps following hepatitis or other hepatic insult)

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[*] or according to the theory of Burnet--all the antibodies for which specific plasma cell clones exist.

by "forbidden clones of immunologically competent cells (I.C.C.) which have lost their normal homeostatic control and produce part of a generalized production of antibodies ones which are destructive to the liver parenchyma[71]". Autoantibodies against the liver itself and kidney have been demonstrated by Gökcen[72] in a number of liver diseases. The production of the rheumatoid factor in lupoid hepatitis according to this theory is incidental.

It might be predicted then that in any analogous "autoimmune" disease with 1) lymphoid infiltration of the diseased organ, 2) the demonstrated occurrence of autoantibodies and 3) the production of an elevated gamma globulin fraction reflecting non-specific antibody production, that the rheumatoid factor will be present. Two diseases which fulfill the above 3 criteria are the Mikulicz-Sjörgen's syndrome[73,74] and Hashimoto's struma[77,80]. The first disease is known to be associated with an extremely high incidence of the rheumatoid factor. Block et al.[73] studied 21 patients with Sjörgen's syndrome and found that 75% of those free of joint involvement had a positive bentonite flocculation test. This is the highest incidence for the rheumatoid factor reported in a non-rheumatoid disease and actually is greater than the incidence seen in rheumatoid arthritis using the same technique[75]. It is interesting also that in Block's patients 71% had anti-nuclear antibodies, 10% showed the LE phenomenon, 14% had thyroglobulin antibodies and 25% exhibited a positive direct Coomb's test. Block and his associates point out that in Sjörgen's syndrome there is a very general and active immunological response.

So far there has been no good study of the incidence of the rheumatoid factor in lymphoid thyroiditis. Selenkow et al.[76] have reported a case showing high levels of circulating antibodies to thyroglobulin and a consistently false-positive test for syphilis but tests for the rheumatoid factor and L.E. factor were negative. Buchanan et al.[81] reported that 3 of 31 women with Hashimoto's disease and no evidence of rheumatoid arthritis had a positive test for the rheumatoid factor. A report on a large series of such patients would be interesting since a high incidence of the rheumatoid factor would be predicted.

That there may be something more going on in these two diseases than the non-specific production of the rheumatoid factor is suggested by the statistical association with rheumatoid arthritis. Heaton[82] analysed 28 cases of Sjörgen's syndrome and found rheumatoid arthritis in 17. Buchanan et al.[81] found clinical and/or radiological evidence of rheumatoid arthritis in 5 of their 31 women with Hashimoto's disease. Although the relationship between these diseases is unclear Waksman[83] suggests, "while it is possible that there are overlapping (cross-reacting) antigens in the different tissues involved in such cases, it is more probable that these represent multiple auto-immunizations occurring in a single individual. Whatever the basic abnormality is, it evidently is present in its most extreme form in patients with 'collagen diseases' and their relatives who are known to make 'antibodies' to a variety of good and poor auto-antigens . . . quite aside from the disease process itself."

Other non-rheumatoid diseases in which an incidence of the

rheumatoid factor greater than 10% has been reported--sarcoidosis[4], syphilis[6], kala-azar[6], lymphomas[18], tuberculosis[18], viral pneumonia[18], leprosy[78], and subacute bacterial endocarditis[18]--cannot be so easily fitted into the class of diseases having an autoimmune component. Most of these are associated with hypergammaglobulinemia, several show lymphoid hyperplasia and at least two (syphilis and viral pneumonia[83]) are known to be associated with the production of auto-antibodies. Postulating that these diseases too produce a temporary derangement of the autoimmune mechanism with resulting non-specific production of gammaglobulins does not seem far-fetched.

The presence of circulating auto-antibodies has been demonstrated in a large number of obviously non-autoimmune disorders e.g., myocardial infarction[83]. It is possible that the production of auto-antibodies (including the non-specific production of the rheumatoid factor) can be a consequence of any non-immunological disease in which there is tissue destruction. The outpouring of cellular debris from necrotic tissue might lead to a temporary immunological dysfunction and auto-immunization. Support for this idea is derived from the observation that among the 25 patients from the group of 500 studied who had the rheumatoid factor but no evidence of rheumatoid disease there is in almost every case some form of active cellular necrosis--mostly hepatocellular damage and metastatic disease but also two cases of iatrogenic myocardial necrosis and one of severe myocardial strain.

Considering the increased incidence of the rheumatoid

factor in liver disease, it should be noted in passing that an association between hepatic dysfunction and rheumatoid arthritis has long been suspected but never proven[69]. A number of older papers by American and English authors* refer to the supposed role of the liver in the production of arthritis. The ideas of a mechanism are vague and at present the liver is not incriminated in articular diseases because the few clinical, pathological and laboratory studies which have been directed to the liver in arthritis have given little or no evidence of a relationship[43,58,59,60,67,68].

As is so often the case in clinical investigation, the incidence of the rheumatoid factor is in large measure a function of the methods used to determine it. This is clearly seen in the increasing incidence reported for rheumatoid arthritis itself as investigators have applied their effort at refining the tests used. Not until the nature of the rheumatoid factor has been worked out by the physical chemists will it be possible to determine the true incidence. At the present time, we are not sure just what is being measured. Presumably, the rheumatoid factor is a class of 19S (or 19S-7S complex which is identified as a 22S peak[51]) macroglobulins with the property of agglutinating latex particles and sheep erythrocytes. Equating this activity with a single, specific "rheumatoid factor" is obviously a hopeless over-simplification. At best, all that can be done at the present is to understand the inadequacy of our knowledge and not expect too much from the limited methods available.

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*cf: The review by P.S. Hench[69].

Information which we have suggests that the rheumatoid factor does not medicate the pathogenesis of rheumatoid disease[2,7]. And the presence of globulins in the serum which will agglutinate latex particles is not tantamount to rheumatoid arthritis. How then, should the occurrence of the "rheumatoid factor" be understood?

Clinically, the results obtained in the several studies reported in this thesis may be viewed in two ways. The first is to consider the presence of "rheumatoid factor", even when present in very small amounts detectable only by the most sensitive methods, as significant. This is an "all-or-none" view. If this position is taken then the occurrence of the rheumatoid factor is certainly wide spread in both rheumatoid and non-rheumatoid disease. Detection of its presence, particularly when very sensitive "one-tube" tests are used will not be diagnostic of anything. What is of greatest importance at present is that although the occurrence of the rheumatoid factor is by no means confined to the rheumatoid diseases, generally the titers present are insignificant outside the rheumatoid group. The Hyland R-A test, particularly when used (as the manufacturer directs) without prior inactivation of "compliment", is extraordinarily sensitive and will produce erroneously large numbers of positive tests for the "rheumatoid factor." Even when the serum is first inactivated it is clear that non-rheumatoid diseases and probably significant numbers of seemingly healthy people will reveal low levels of agglutinating activity.

The second manner in which to view these studies on the incidence of the rheumatoid factor is to admit, as several authors have already stated[19,20,21,22], that the presence of rheumatoid factor as we detect it today is not diagnostic of any specific disease entity and then to ignore this as the result of highly sensitive but not very specific methods. Then the fact that only rarely are significant titers of the rheumatoid factor found in non-rheumatoid disease becomes important. Liver disease does seem to have a special place. But even here the titers are generally quite low or negative in spite of the increased incidence. What is then demanded for the diagnosis of a rheumatoid disease is not a positive test for the rheumatoid factor but a sufficiently elevated (1:320) titer. Craig et al.[20] sum up this view with the observation that ". . . an elevated titer is not diagnostic of any single disease entity . . . nevertheless (it is) encountered with sufficient rarity in diseases unrelated to the so-called 'collagen' group that it can, on occasion, be a significant observation added to other evidence pointing in the direction of connective tissue disease."

APPENDIX

Use of the Euglobulin Fraction

Precipitation of the euglobulin fraction is an attempt at concentrating and purifying the rheumatoid factor. Presumably this should increase the sensitivity of the titering and, perhaps, unmask hitherto undetectable amounts of the rheumatoid factor. Ziff et al.[23] reported a decrease in the number of false-positive reactions when the euglobulin fraction was used supposedly due to the elimination of non-specific agglutinating factors.

In this thesis, euglobulin precipitation was done to see what activity might be found in the sera of patients with sarcoidosis and liver disease. It was unknown whether use of such a method would increase the incidence of the rheumatoid factor by detecting small amounts or actually decrease it by the elimination of non-specific agglutination.

The method used is that developed by Howell et al.[36], Craig et al.[20] and Kirby[38] after the preliminary work of Neurath and associates[39] and Hall et al.[8] The basic mechanism is the precipitation of an active serum euglobulin fraction by isoelectric precipitation in the cold. This was done originally by dialysing the serum against dilute buffer[56]. Howell et al.[36] and Craig et al.[20] substituted rapid dilute hydrochloric acid precipitation for the lengthy dialysis.

The method employed in this thesis is essentially that described in the paper by Craig, Kirby and Persons[20]. Serum,

either fresh or frozen, is incubated at 56°C. for 30 minutes to inactivate non-specific agglutinating substances. To 1.0 ml of this inactivated serum are added slowly down the side of the test tube 9 ml of distilled water (Cutter's Distilled Water for Injection USP) made slightly acid (pH 6.1-6.4) by the addition of a very small amount of dilute hydrochloric acid. The mixture is then incubated in an ice water bath at 4-8°C. for 1-2 hours. This generally, but not invariably, produces a faint cloudiness. The euglobulin precipitate is then centrifuged in a refrigerated Servall centrifuge at 3,000 rpm for 10 minutes. The supernatant is poured off and the tubes drained for a few minutes by inverting over filter paper. The precipitate is then dissolved in 1.0 ml of physiological saline. Warming in a 56°C. bath aids this. Any insoluble material is precipitated by again centrifuging in the Servall at 2,000 rpm for 10 minutes. The supernatant containing the euglobulin is often opalescent. This is then used for titering.

Typical results, comparing the titers of the euglobulin fraction obtained in the manner described with titering whole serum, are given below.

		whole serum	euglobulin
Fenn	B69388	1:1280	1:640
Carofalo	487646	1:2560	1:640
Comston	379534	1:1280	1:640
Gardo	380562	1:640	1:160
Cannon	A14505	1:320	Neg

Consistently the euglobulin fraction produced lower titers than when whole serum was used. According to the developers, there should be increased sensitivity when the euglobulin fraction is used. In patients with definite and classical rheumatoid

arthritis, for example, Howell et al.[36] found 84% positive with whole serum and 95% positive using the euglobulin fraction in a single tube test. It doesn't necessarily follow, however, that increased sensitivity will be reflected in increasing titers of serum already having significant titers but one might expect this to be the case. The euglobulin method is said to "compare favorably[36]" in its specificity with whole serum. Ziff et al.[23] believes that the specificity is significantly increased by eliminating non-specific agglutinating factors. Cathcart et al.[78] were able to increase the positive tests for the rheumatoid factor in the sera of lepers from 24 to 44% by using a euglobulin fraction. Unfortunately the data in this thesis are inadequate to provide an answer to the question of whether the use of a euglobulin fraction increases the specificity. From the number of negative titers using euglobulin following positive screen tests and titers of the whole serum in non-rheumatoid diseases, the indications are that it may. What is abundantly clear from the work presented here is the observation which Howell et al.[36] found "disconcerting" that the Hyland screening test is exquisitely sensitive. They found a 5 fold greater frequency of positive reactions to the macroscopic slide test than to the euglobulin latex method in a variety of non-rheumatoid diseases--a finding duplicated in this thesis. The point is that agglutinating substances seem to be produced in a variety of diseases and while the Hyland test is extraordinarily sensitive the price is its low specificity for rheumatoid disease.

SUMMARY

Several groups of patients were studied to determine the occurrence of the rheumatoid factor in non-rheumatoid disease. Using the Hyland R-A macroscopic slide test to screen fresh serum positive reactions were widespread and non-specific. When the serum was inactivated at 56°C. for 30 minutes prior to screening it became more specific. Of 500 ill patients 7.6% had positive tests using inactivated sera. Half of these represented patients with a rheumatoid disease. Of 461 patients in the group judged free of a rheumatoid-collagen-arthritic disease, 5.4% had serum capable of agglutinating the latex particles following heat inactivation. In almost half of these there was documented or highly probable liver disease. One-forth had metastatic disease. The titers of the agglutinating activity in these patients with non-rheumatoid disease were generally low or negative compared with high titers in the patients with rheumatoid disease. It is stressed that while the incidence of the rheumatoid factor in non-rheumatoid disease is low (5%), it is associated in large measure with liver disease where the incidence is 20-30%. Also, the titers present in non-rheumatoid disease are characteristically very low. Patients with hyperglobulinemia were studied and, in general, did not have a positive screening for the rheumatoid factor unless rheumatoid or hepatic disease was present. It is noted that hyperglobulinemic sera frequently contains heat-labile, non-specific agglutinating activity. Study of 8 patients with

biopsy proven sarcoidosis showed only one with a titerable amount of the rheumatoid factor and this was quite low (1:160). A group of 30 patients with documented liver disease was studied. The incidence of a positive latex agglutinating screening test using inactivated serum was 33%. Only four of these patients had titerable amounts of agglutinating activity in the serum and here too the titers were characteristically low. Throughout these studies, the presence of hypergammaglobulinemia was consistently seen when the rheumatoid factor was present, but no consistent correlation between an elevation in this fraction and the presence of agglutinating activity could be demonstrated.

The Hyland R-A latex F II slide test is shown to be exquisitely sensitive--many times more so than euglobulin precipitation--but it is extremely non-specific even when inactivated serum is used. Only half the positive tests occur in rheumatoid disease. Titering of the euglobulin fraction of patients with liver disease and sarcoidosis was generally negative although information is inadequate for a fair appraisal.

The production of the rheumatoid factor in liver diseases is probably part of a generalized and non-specific amnestic production of globulin by the round cell infiltrate in the diseased liver. It is suggested that when the rheumatoid factor is present in a non-rheumatoid disease there is cellular necrosis with the release of intra-cellular debris. A temporary derangment of the immunological apparatus occurs with production of a potpourri of circulating antibodies and non-specific gamma globulins. Using this hypothesis, the occurrence of the rheumatoid

factor in "plasma cell hepatitis," Sjögren's syndrome and Hashimoto struma is discussed.

The fact that the presence of the rheumatoid factor as it is detected at present is not diagnostic of any specific disease entity is recognized. However, emphasis for diagnosis is placed on the demonstrated fact that only rarely are elevated titers ($\geq$1:320) of agglutinating activity found in the non-rheumatoid diseases.

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