DEMONSTRATION OF CIRCULATING ANTINUCLEAR GLOBULINS IN ULCERATIVE COLITIS *

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(Submitted for publication April 20, 1961; accepted May 29, 1961)

Although Andresen (1) first considered "allergy" as a factor in the pathogenesis of ulcerative colitis in 1925, the recent studies of Levine, Kirsner, Klotz and Elchlepp (2–4) have renewed interest for the role of hypersensitivty in this disorder. Various clinical features suggest the presence of an altered immune state in ulcerative colitis. Among these are sensitivity to drugs and blood transfusion (5), the beneficial effects of ACTH and corticosteroids (6) and such extracolonic manifestations as erythema nodosum (7), uveitis (8), hepatitis (9, 10), myocarditis (6), glomerulitis (5, 11), arthritis (12–14), purpura (5), hemolytic anemia with positive Coombs test (15), leukopenia (16) and splenomegaly (8). In addition, a few reports have called attention to the presence of ulcerative colitis in patients with lupus erythematosus (17), scleroderma (18), or periarteritis nodosa (19).

Previous studies have shown the presence of circulating antinuclear globulins in patients with systemic lupus erythematosus as well as in cases of rheumatoid arthritis, complicated by splenomegaly and leukopenia (Felty's syndrome) (20). In the course of testing sera from patients presenting this clinical picture, two individuals with ulcerative colitis were found to have circulating antinuclear factors. The present study was undertaken to determine the nature and frequency of antinuclear globulins in ulcerative colitis. A preliminary report has been published (21).

MATERIALS AND METHODS

Human sera. Sera were obtained from 24 patients with proven ulcerative colitis and from 13 individuals who had previously undergone colectomy for this disorder. Four patients were studied before and after colectomy and are included in both groups. Over 300 sera from normal subjects and from patients with various disease states were tested while the study was in progress. Whenever multiple tests were performed, aliquots of the same serum sample were used. All sera were stored at −20° C.

Fluorescent antiglobulin tests. The details of the technique used have been reported previously (20). Briefly, the test sera were superimposed for 1 hour on: *1)* normal human peripheral blood smears fixed in 95 per cent ethanol (20), and on *2)* nucleohistone spots prepared from calf thymocytes (22, 23). The slides were thoroughly washed and fluorescein-labeled rabbit antihuman γ-globulin, absorbed with rat liver powder, was then used as a histochemical stain for human γ-globulin. The first procedure tests the affinity of the sera for human leukocyte whole nuclei and the fluorescent label is detected by ultraviolet microscopy. In the second method, the sera are tested against calf nucleoprotein and fluorescence is visible to the naked eye when the slides are exposed to the beam of a Woods lamp.

Lupus erythematosus (LE) cell preparations. The technique used was a variation of the Zinkham-Conley method (24). One ml of heparinized normal blood was added to 0.5 ml of test serum. After standing at room temperature for 2 hours with occasional shaking, 1.5 ml of the mixture was transferred to screw-top tubes containing glass beads and rotated at 40 rpm for 30 minutes at 37° C. The contents were then centrifuged in small-bore tubes to isolate the buffy coat which was smeared on glass coverslips, stained with Wright-Giesma and examined microscopically for the presence of LE cells.

Rheumatoid factor tests. Commercially available human γ-globulin-coated latex particles (Hyland RA test) were mixed with a 1:20 dilution of the patient's serum on a glass slide. Visible flocculation constituted a positive result.

Thyroglobulin antibodies. Commercially available thyroglobulin-coated latex particles (Hyland TA test) were mixed on glass slides with test sera. The sera were previously incubated at 56° C for 30 minutes and tested undiluted and at a 1:20 dilution. Macroscopic clumping constituted a positive result.

Serum protein determinations. Filter paper electrophoresis was performed in veronal buffer at pH 8.6 in a model R Spinco apparatus. The strips were stained with bromphenol blue and scanned with the Analytrol photo-

* Supported in part by United States Public Health Service Grants OG-14, H-1577, C-2578, CY-5944, A-1785, A-4759, and A-3473, and a grant from the Connecticut Branch of the American Cancer Society.

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TABLE I

Clinical and laboratory findings in patients with ulcerative colitis

Patients		Age, Sex	Duration of disease	ACTH-steroid therapy	Serum proteins A/G	Thyroglobulin antibody	Rheumatoid factor	LE prep.	Fluorescent antiglobulin test: Nucleoprotein	Fluorescent antiglobulin test: Whole nuclei	Remarks
1.	C.F.	27 ♀	8 yrs	Yes	2.6/2.8	0	0	0	0	+	Arthritis, splenomegaly, leukopenia
2.	J.L.	38 ♂	7 yrs	0	2.3/3.1	0	0	0	0	+	Arthritis, splenomegaly, leukopenia
3.	L.D.	40 ♂	23 yrs	0	2.48/2.64	0	0	0	0	+	Eosinophils 6%
4.	M.J.	45 ♀	4 mos	Yes	2.2/3.2	0	0	0	0	0	Alopecia
5.	M.S.	26 ♀	4 yrs	Yes	2.9/3.6	0	0	0	0	+	Patient and mother had rheumatic fever
6.	E.M.	55 ♀	2 mos	Yes	1.74/3.31	0	0	0	0	+	Disease developed after penicillin treatment
7.	B.A.	23 ♂	4 yrs	Yes	2.74/3.87	0	0	0	0	0	
8.	F.P.	17 ♂	1 yr	0	2.08/3.78	+	0	0	0	+*†	Hepatitis, arthritis. Aunt and cousin, ulcerative colitis
9.	J.G.	46 ♂	10 yrs	0	3.80/2.89	0	0	0	0	+	Psoriasis
10.	R.F.	28 ♂	8 yrs	Yes	1.55/4.14	0	0	0	0	+	Arthritis, splenomegaly, pleurisy, eosinophils 5%
11.	R.G.	19 ♂	1 yr	Yes	2.8/3.7	0	0	0	0	0	Arthritis, hepatitis, recurrent thrombophlebitis

* Test negative after ACTH-steroid therapy.
† Test negative after colectomy.

TABLE I—(*Continued*)

Patients		Age, Sex	Duration of disease	ACTH-steroid therapy	Serum Proteins A/G	Thyroglobulin antibody	Rheumatoid factor	LE prep.	Fluorescent antiglobulin test: Nucleoprotein	Fluorescent antiglobulin test: Whole nuclei	Remarks
12.	F.M.	35♂	2.5 yrs	0		0	0	0	0	+	Past history of arthralgias and hives
13.	R.M.	23♀	1 yr	Yes	2.3/4.2	0	0	0	0	+†	Allergic to penicillin
14.	J.H.	27♀	2.5 yrs	Yes	3.1/2.86	0	0	0	0	0	Arthritis, erythema multiforme nodosum
15.	M.H.	65♀	10 days	0	2.2/3.6	0	0	0	0	0	Leukopenia, eosinophils 6%
16.	J.P.	16♂	1 yr	Yes	2.73/5.76	0	0	0	0	+*	Dermatitis, penicillin allergy, splenomegaly, thromboses, pyroderma gangrenosum, glomerulitis, eosinophils 15%
17.	M.W.	45♂	15 yrs	0	3.72/3.66	0	0	0	0	+	Arthralgias, regional ileitis. Son, rheumatic fever
18.	A.L.	45♂	2 yrs	0	1.55/3.22	0	0	0	0	0†	Butterfly rash
19.	J.G.	42♂	25 yrs	0	3.80/3.32	0	0	0	0	+	Brother, rheumatic heart disease. Sister, nephritis
20.	C.M.	19♂	9 yrs	Yes	2.30/3.32	0	0	0	0	+	Arthritis. Mother, rheumatic heart disease
21.	M.W.	37♀	1 yr	0		0	0	0	0	+	
22.	S.R.	61♂	14 days	0	3.72/4.68	+	0	0	0	+	
23.	C.S.	57♂	1 yr	Yes		0	0	0	0	+	
24.	S.G.	18♂	2 yrs	Yes	2.82/3.73	0	0	0	0	+†	

densitometer. Normal values for this laboratory were: albumin, 3.39 to 5.59 g; globulin, 1.88 to 4.08 g.

RESULTS

The pertinent clinical and laboratory findings in 24 patients with ulcerative colitis are listed in Table I. Positive fluorescent antiglobulin tests against whole nuclei were observed in 18 of these patients (Figure 1A). However, uniformly negative results were obtained when sera were tested for affinity to calf thymus nucleoprotein (Figure 2), for the presence of the rheumatoid factor or for their ability to induce LE cells. Thyroglobulin antibody tests were negative in all but two individuals, both without evidence of thyroid disease. Serum protein determinations indicated borderline elevation of serum globulins in four subjects and frequent hypoalbuminemia, a common finding in ulcerative colitis. Positive antinuclear tests were not related to age, sex or history of hypersensitivity disorders, although frequent manifestations of the latter were present in several of the patients or their relatives. Approximately half of the patients were receiving ACTH or corticosteroid therapy at the time of testing, and in one-third the disease had been present for over 5 years.

The results of the fluorescent antinuclear test with respect to certain clinical findings are reported in Table II. The over-all results of the test in this series of patients did not seem to be greatly influenced by ACTH or corticosteroid therapy. Nine of 13 individuals under treatment had positive tests as compared with 9 of the 11 patients not receiving hormones. However, in two individuals (Table I, Subjects 8 and 16), the fluorescent antinuclear tests became negative during hormone therapy. In 9 of 13 postcolectomy patients, including two individuals who had positive tests before operation and another who had reverted to negative on hormone therapy, no antinuclear globulins were detected (Table I). In the group of patients whose disease manifestations

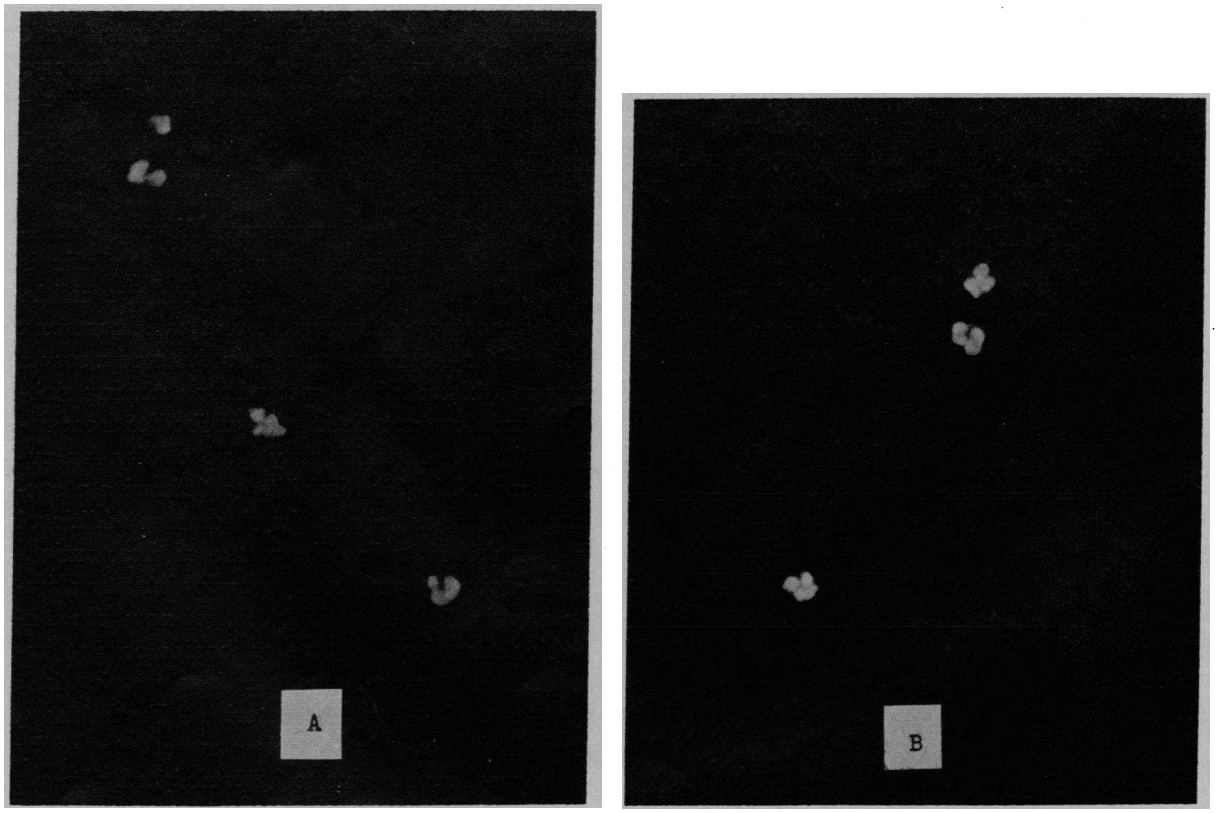

FIG. 1. A) POSITIVE FLUORESCENT ANTIGLOBULIN TEST AGAINST HUMAN LEUKOCYTE WHOLE NUCLEI PREVIOUSLY EXPOSED TO SERUM OF A PATIENT WITH ULCERATIVE COLITIS. B) SIMILAR REACTION WITH SERUM FROM A PATIENT WITH LUPUS ERYTHEMATOSUS. Note brighter fluorescence.

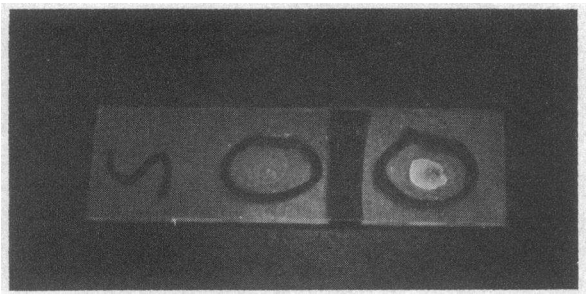

FIG. 2. FLUORESCENT ANTIGLOBULIN TEST AGAINST CALF THYMUS NUCLEOPROTEIN SPOTS PREVIOUSLY EXPOSED TO TEST SERA. Negative reaction (left) with ulcerative colitis serum; positive reaction (right) with lupus erythematosus serum.

had been present for less than 5 years, ten had positive and six had negative antinuclear tests as compared with the eight individuals with a history of over 5 years' duration, all of whom were found to have circulating antinuclear globulins. All four patients with splenomegaly and seven of the nine who suffered joint manifestations had positive antinuclear tests.

Table III lists the results obtained to date with sera tested for antinuclear factors from a group of normal controls and from patients with a variety of disease states. Certain specific disorders, included in this list for comparison, have formed the basis for or have been included in part in earlier reports and are designated by appropriate references (20, 25–27). In every case, the sera studied for affinity to calf thymus nucleoprotein were also tested against fixed whole nuclei of normal peripheral blood leukocytes, but in some cases only the latter test was performed. Circulating antinuclear factors have been detected by these methods in lupus erythematosus, rheumatoid arthritis and related disorders, as well as in chronic liver disease and in some drug reactions.

TABLE II

Correlation of antinuclear test with clinical findings in ulcerative colitis

Clinical finding	Fluorescent antiglobulin test (whole nuclei)	
	No. positive	No. negative
Arthritis and arthralgia	7	2
Splenomegaly	4	0
Duration of disease		
Under 5 yrs	10	6
Over 5 yrs	8	0
Postcolectomy	4	9
ACTH-steroid therapy		
Yes	9	4
No	9	2

DISCUSSION

In addition to the clinical observations previously mentioned, some support for a possible relationship of ulcerative colitis to the hypersensitivity states stems from a few recent experimental studies. Broberger and Perlmann (28) demonstrated that sera from patients with ulcerative colitis contained precipitating and hemagglutinating factors capable of reacting with tissue extracts from colon as well as from liver or kidney. Furthermore, the same authors observed absorption of γ-globulins from these sera onto colonic cells in tissue culture by fluorescent techniques, but did not describe nuclear or cytoplasmic localization, specificity, or prevalence of this reaction. Polcak and Vokurka (29) studied a circulating factor which agglutinated collodion particles coated with extracts of normal colonic mucosa and submucosa. Bregman and Kirsner (30) report detecting circulating antibodies apparently specific for colon mucosa by double agar diffusion and hemagglutination techniques.

The results of the present investigation demonstrate that a circulating antinuclear γ-globulin is present in many patients with ulcerative colitis. It is important to emphasize that the fluorescent antiglobulin technique, as used in this study, serves only as a histochemical method for detecting globulins and does not distinguish antibodies from abnormal proteins which may have affinity for nuclear material. The inability to determine a specific antigen, as well as the absence of additional information regarding the nature of these antinuclear globulins and their behavior in other immunological systems, precludes, for now, the conclusion that they are true antibodies or that immunological mechanisms are involved in their reactions with nuclear material. Furthermore, the relation of this factor to those detected by other workers remains to be established. In our system this factor was detected on homologous leukocyte nuclei in a reaction quantitatively similar to that observed with sera of some patients with

TABLE III

Results of tests for antinuclear factors in sera of individuals without ulcerative colitis

Diagnosis	Fluorescent antibody test			
	Nucleoprotein		Whole nuclei	
	No. tested	No. positive	No. tested	No. positive
Systemic lupus erythematosus (20)	12	12	21	21
Rheumatoid arthritis (25)	50	5	50	17
Other collagen diseases	5	2	5	5
Hepatitis and cirrhosis (26)	19	3	65	24
Leukemias and lymphomas (27)	30	0	30	0
Drug reactions	7	0	7	2
Carcinoma of colon	17	0	17	0
Other diseases of colon*	9	0	9	1†
Other diseases (20)	62	0	108	0
Normal laboratory personnel	15	0	15	0
Totals	226	22	327	70

* Tests negative in: amebic colitis, 2; mucous colitis, 2; diverticulitis, 2; schistosomiasis, 1; multiple polyps, 1.
† Positive antinuclear test in man with familial polyposis whose son has ulcerative colitis.

rheumatoid arthritis (25), chronic hepatitis (26), drug reaction, scleroderma, polyarteritis, and in the sera of patients with lupus erythematosus in relative remission. Higher titers of this factor are usually encountered in patients with rheumatoid arthritis accompanied by complications such as splenomegaly and leukopenia, and in individuals with active lupus erythematosus (20, 25). In over 250 sera from healthy subjects as well as from patients with other diseases characterized by leukopenia, hyperglobulinemia, splenomegaly or colon pathology, antinuclear globulins were not demonstrable.

While many of our patients exhibited some of the features often attributed to hypersensitivity disorders, none fulfilled the clinical picture of systemic lupus erythematosus. The LE cell preparations, in agreement with the findings of Lagercrantz, Winberg and Zetterström (5), were uniformly negative. The antinucleoprotein test, which has shown closer correlation with the LE phenomenon than the reaction against whole nuclei (25), was also negative in every case. In accord with other reports (12, 13), the rheumatoid factor was absent in all of our patients with ulcerative colitis even when arthritis was present. Thyroglobulin antibodies were present in two of our patients, but this finding has been reported in 18 per cent of a normal hospital population (31).

Although the number of cases is too small to warrant definite conclusions, attention should be called to the fact that patients with splenomegaly, arthritis or arthralgia, or disease of over 5 years' duration usually had positive antinuclear tests (Table II). While, in general, postcolectomy cases gave negative results, this serum factor was present in nine patients on ACTH-corticosteroid therapy as well as in nine patients not receiving hormones. At the present time we do not know what may be spontaneous fluctuations of these factors during the natural course of the disease, the influence of hormones, or the role played by the presence of a diseased colon.

The possible implications of the presence of circulating antinuclear globulins in ulcerative colitis should be tempered by the realization that little is known about their significance or about the nature of the diseases in which they are present. No one has yet demonstrated that these abnormal globulins play a specific role in the pathogenesis of any of the disorders in this group (32). A recent report (33) that clinically healthy relatives of patients with lupus erythematosus have circulating antinuclear globulins suggests the possibility that this is a genetically controlled protein abnormality which merely denotes or accompanies an unusual type of immunological reactivity. In some individuals with this constitution, exposure to unknown stimuli might result in different clinical syndromes with some common features.

Whether this circulating antinuclear globulin in the individual with ulcerative colitis represents an increased susceptiblity for, a primary factor in, or

an incidental result of the disease process remains to be determined.

SUMMARY

Sera from 24 patients with ulcerative colitis were studied for the presence of antinuclear globulins by the fluorescent antiglobulin technique. In 18 patients (75 per cent) a positive reaction was detected on whole nuclei of human leukocytes, but no affinity for calf thymus nucleoprotein was observed. Tests for rheumatoid factor and lupus erythematosus cell induction were negative.

Antinuclear factors were usually present in patients with splenomegaly, arthritis or arthralgia and long-standing disease (over 5 years). They were absent in 9 of 13 patients who had previously undergone colectomy for their disorder, including 2 who had positive tests before surgery. While positive antinuclear tests were present in 9 patients on ACTH-corticosteroid therapy, in 2 the test became negative during treatment.

Over 300 sera from normal subjects and patients with various diseases were tested. Positive antinuclear tests were encountered in systemic lupus erythematosus, rheumatoid arthritis and related collagen disorders, as well as in chronic hepatitis, cirrhosis and in some drug reactions.

The presence of a circulating antinuclear globulin in ulcerative colitis is of interest, since many patients with this disease exhibit features often attributed to hypersensitivity disorders.

ACKNOWLEDGMENT

We are grateful to Dr. S. C. Finch for his support and interest during this study and to Mr. Harvey Sage and Mrs. Bette Epstein for technical assistance.

REFERENCES

1. Andresen, A. F. R. Gastrointestinal manifestations of food allergy. Med. J. Rec. 1925, **122**, 271, (suppl.).
2. Levine, M. D., Kirsner, J. B., and Klotz, A. P. A new concept of the pathogenesis of ulcerative colitis. Science 1951, **114**, 552.
3. Kirsner, J. B., and Elchlepp, J. The production of an experimental ulcerative "colitis" in rabbits. Trans. Ass. Amer. Phycns 1957, **70**, 102.
4. Kirsner, J. B. Editorial: Ulcerative colitis—A challenge. Arch. intern. Med. 1958, **101**, 3.
5. Lagercrantz, R., Winberg, J., and Zetterström, R. Extra-colonic manifestations in chronic ulcerative colitis. Acta paediat. (Uppsala) 1958, **47**, 675.
6. Kirsner, J. B., Palmer, W. L., Spencer, J. A., Bicks, R. O., and Johnson, C. F. Corticotropin (ACTH) and the adrenal steroids in the management of ulcerative colitis: Observations in 240 patients. Ann. intern. Med. 1959, **50**, 891.
7. Foster, J. J., and Brick, I. B. Erythema nodosum in ulcerative colitis. Gastroenterology 1954, **27**, 417.
8. Bargen, J. A. Complications and sequelae of chronic ulcerative colitis. Ann. intern. Med. 1929, **3**, 335.
9. Jones, G. W., Baggenstoss, A. H., and Bargen, J. A. Hepatic lesions and dysfunction associated with chronic ulcerative colitis. Amer. J. med. Sci. 1951, **221**, 279.
10. Gray, N., Mackay, I. R., Taft, L. I., Weiden, S., and Wood, I. J. Hepatitis, colitis, and lupus manifestations. Amer. J. dig. Dis. 1958, **3**, 481.
11. Jensen, E. J., Baggenstoss, A. H., and Bargen, J. A. Renal lesions associated with chronic ulcerative colitis. Amer. J. med. Sci. 1950, **219**, 281.
12. Bywaters, E. G. L., and Ansell, B. M. Arthritis associated with ulcerative colitis: A clinical and pathological study. Ann. rheum. Dis. 1958, **17**, 169.
13. Wright, V., and Watkinson, G. The arthritis of ulcerative colitis. Medicine (Baltimore) 1959, **38**, 243.
14. Fernandez-Herlihy, L. The articular manifestations of chronic ulcerative colitis: An analysis of 555 cases. New Engl. J. Med. 1959, **261**, 259.
15. Lorber, M., Schwartz, L. I., and Wasserman, L. R. Association of antibody-coated red blood cells with ulcerative colitis; report of four cases. Amer. J. Med. 1955, **19**, 887.
16. Levine, M. L., Miranda, M., Engle, R. L., Jr., and Almy, T. P. Leukocyte response in nonspecific ulcerative colitis (abstract). Gastroenterology 1960, **38**, 971.
17. Brown, C. H., Haserick, J. R., and Shirey, E. K. Chronic ulcerative colitis with systemic lupus erythematosus: Report of a case. Cleveland Clin. Quart. 1956, **23**, 43.
18. Bicks, R. O., Goldgraber, M. B., and Kirsner, J. B. Generalized scleroderma associated with chronic ulcerative colitis. Amer. J. Med. 1958, **24**, 447.
19. Wasserman, F., Krosnick, A., and Tumen, H. Necrotizing angiitis associated with chronic ulcerative colitis. Amer. J. Med. 1954, **17**, 736.
20. Calabresi, P., Edwards, E. A., and Schilling, R. F. Fluorescent antiglobulin studies in leukopenic and related disorders. J. clin. Invest. 1959, **38**, 2091.
21. Thayer, W., Spiro, H. M., and Calabresi, P. Antinuclear globulins in ulcerative colitis. Clin. Res. 1960, **8**, 369.
22. Crampton, C. F., Lipshitz, R., and Chargaff, E. Studies on nucleoproteins. I. Dissociation and reassociation of the deoxyribonucleohistone of calf thymus. J. biol. Chem. 1954, **206**, 499.
23. Friou, G. J. Clinical application of a test for lupus globulin-nucleohistone interaction using fluorescent antibody. Yale J. Biol. Med. 1958, **31**, 40.

24. Zinkham, W. H., and Conley, C. L. Some factors influencing the formation of L.E. cells. Bull. Johns Hopk. Hosp. 1956, **98**, 102.
25. Calabresi, P., Friou, G., and Finch, S. Characterization of rheumatoid states by study of antinuclear factors. Clin. Res. 1960, **8**, 197.
26. Calabresi, P., and Greenberg, M. Circulating antinuclear globulins in patients with chronic liver disease (abstract). J. clin. Invest. 1960, **39**, 976.
27. Calabresi, P., and Finch, S. The value of the fluorescent antiglobulin reaction in the differential diagnosis of aleukemic leukemia *in* Proceedings of the Eighth International Congress of Blood Transfusion. Tokyo, 1960.
28. Broberger, O., and Perlmann, P. Autoantibodies in human ulcerative colitis. J. exp. Med. 1959, **110**, 657.
29. Polcak, J., and Vokurka, V. Auto-immune reactions in the course of ulcerative colitis. Amer. J. dig. Dis. 1960, **5**, 395.
30. Bregman, E., and Kirsner, J. B. Colon antibodies in ulcerative colitis (abstract). J. Lab. clin. Med. 1960, **56**, 795.
31. Hackett, E., Beech, M., and Forbes, I. J. Thyroglobulin antibodies in patients without clinical disease of the thyroid gland. Lancet 1960, **2**, 402.
32. Holman, H. R. Editorial: Systemic lupus erythematosus—Disease of an unusual immunologic responsiveness? Amer. J. Med. 1959, **27**, 525.
33. Pollak, V. E., Mandema, E., and Kark, R. M. Antinuclear factors in the serum of relatives of patients with systemic lupus erythematosus. Lancet 1960, **2**, 1061.