RHEUMATOID FACTOR AND THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

By ROBERT C. MELLORS, M.D., ADAM NOWOSLAWSKI,* M.D., LEONHARD KORNGOLD, PH.D., AND BETH L. SENGSON, D.D.S.

(From the Hospital for Special Surgery, Philip D. Wilson Research Foundation, affiliated with The New York Hospital–Cornell Medical Center, New York)

PLATES 49 TO 54

(Received for publication, October 13, 1960)

In previous work (1) fluorescein-labeled, aggregated human γ-globulin was demonstrated to be a sensitive reactant for the detection of rheumatoid factor in frozen sections of synovial membranes and lymph nodes obtained from patients with active rheumatoid arthritis. Observations of similar kind have been made with fluorescent antigen-rabbit antibody (immune) complex and are reported here. Thus in analogy with the precipitin test and the sheep cell agglutination reaction for rheumatoid factor (2), two categories of fluorescent reactants have been prepared for the detection of rheumatoid factor in tissue sections.

Materials and Methods

Fluorescent Immune Complex and Other Fluors.—The starting material for the preparation of the fluorescent complex was rabbit antiserum against bovine serum albumin. The rabbits were immunized by intramuscular injections of crystalline bovine serum albumin (Armour Laboratories) incorporated in complete Freund adjuvant (Difco Laboratories). Each of six New Zealand brown rabbits received an initial injection of 10 mg. bovine serum albumin, followed by a similar injection 1 week later. Three weeks after the second injection test bleedings were made. All samples of serum gave a strong precipitin reaction in agar gel with bovine serum albumin at a concentration of 400 μg. per ml. The rabbits were exsanguinated, and the antisera were pooled. Quantitative precipitin reactions were then carried out with aliquots of the pooled antiserum, and the zone of equivalence was determined with unlabeled and fluorescein isothiocyanate–labeled (3) bovine serum albumin. Immune precipitates were formed by the addition of labeled antigen at equivalence, harvested by centrifugation after several days' incubation in the cold, washed several times with chilled saline, and then rendered in part soluble by the addition of labeled antigen in twice the equivalent amount (4). After several days' equilibration in the cold, the soluble complex was obtained in the supernatant fluid by centrifugation. This comprised the fluorescent immune complex, to be used as a staining reactant for rheumatoid factor. An immune complex was similarly prepared with unlabeled antigen for control staining procedures. As shown by the Ouchterlony technique, both the labeled and the unlabeled immune complex formed zones of precipitate with sera from patients with rheumatoid arthritis.

* Rockefeller Foundation Fellow. Present address: Department of Pathologic Anatomy, Medical Academy, Warsaw, Poland.

Fluorescein-labeled aggregated human γ-globulin and fluorescent antibody for 19S human γ-globulin were prepared as previously described (1) and were tested for their specificity.

Source and Nature of Tissues.—Tissues were obtained from 7 patients: 5 adults, 4 females and 1 male, ranging in age from 31 to 69 years, with classical active rheumatoid arthritis, of 7 to 13 years' duration, 1+ to 4+ latex fixation tests, and systemic treatment with corticosteroids for several years; and 2 children, a boy, age 8, and a girl, age 9, with juvenile rheumatoid arthritis of ½ and 5 years' duration respectively, − and ± latex fixation tests, and no systemic treatment with corticosteroids. Diagnostic biopsies and surgical procedures performed on these patients by the hospital staff provided the following specimens for study: synovial and capsular tissues of knee, 4, including a preoperative needle biopsy and a total synovectomy in an adult male, a total synovectomy in an adult female, and a synovial biopsy in a girl; synovial tissue of wrist, 1, in an adult female; and lymph nodes, 3, of which two were obtained from adult females and one from a girl. Tissue blocks, numbering 127, were cut from these specimens, frozen for special study, and fixed and embedded in paraffin for routine study in the following number: synovial tissue, special 52, routine 54; and lymph nodes, special 9, routine 12. The pathological diagnosis of synovial tissue was in each case either chronic or subacute exudative and proliferative synovitis histologically compatible with rheumatoid arthritis. The lymph nodes were in each instance moderately hyperplastic.

Control material from 12 patients, 18 to 61 years of age, 6 males and 6 females, without rheumatoid arthritis provided the following tissue blocks for study: synovial tissue, special 34, routine 42; and lymph nodes, special 5, routine 4. Among the synovial tissues were surgical specimens obtained from patients with degenerative and traumatic osteoarthritis, infectious, tuberculous, and gouty arthritis, pigmented villonodular synovitis, synovial chondromatosis, Reiter's syndrome, and Boeck's sarcoid. The lymph nodes were derived from two sources: biopsies of hyperplastic and normal nodes, and autopsies in an example each of Waldenström's macroglobulinemia (5) and Boeck's sarcoid.

In the present study approximately 400 frozen sections, treated with fluors, were examined and described and 500 individual photomicrographs were made as a permanent record of the observations. This work comprised a part of a continuing investigation of rheumatoid arthritis in which to date more than 1200 immunofluorescence reactions have been carried out on tissue sections.

Preparation of Tissue Sections.—Frozen sections of unfixed tissues were cut in a microtome cryostat at an indicated thickness of 4 μ as previously described (1). On rare occasion, fresh tissue blocks with a volume of about 1 mm.^{3} were frozen at the temperature of liquid nitrogen, dried in the frozen state, embedded with diethylene glycol distearate (6), and sectioned with a glass knife at 1 to 2 μ with a Servall Porter-Blum microtome.

Fluorescence Staining Procedures.—The general steps in the procedures, including the absorption of fluors with rabbit bone marrow powder, were carried out as described elsewhere (1). Fluorescent immune complex and, for correlative work, fluorescent aggregated human γ-globulin (1), were used, with the appropriate control procedures noted below, to detect rheumatoid factor; and fluorescein-labeled rabbit antibody of known immunospecificity (1) was used to detect 19S human γ-globulin. Staining reactions were carried out for 1 hour at room temperature.

Control Procedures.—The specificity of the apple-green staining patterns in tissue sections treated with fluorescent immune complex and attributed to the presence of rheumatoid factor was confirmed by inhibition of the reactions with (*a*) unlabeled rabbit antibody against 19S human γ-globulin, (*b*) unlabeled immune complex, and (*c*) unlabeled aggregated human γ-globulin. In these inhibition reactions the sections were exposed to the appropriate protein solution for 2 hours at room temperature, followed by washing in buffered saline and staining with the fluorescent immune complex. The lack of influence of complement on the staining

reactions was shown by the treatment of tissue sections with heat (56°C. for ½ hour) and by the use of fluorescent immune complex prepared from complement-inactivated rabbit antiserum against bovine serum albumin, neither of which altered the results.

The specificity of the staining patterns obtained with fluorescent aggregated human γ-globulin and attributed to the presence of rheumatoid factor was confirmed, as before (1), by inhibition with either (*a*) unlabeled rabbit antibody against 19S human γ-globulin or (*b*) unlabeled aggregated human γ-globulin. Prior treatment of sections with unlabeled immune complex inhibited, variously, none or some of the staining reaction with fluorescent aggregated human γ-globulin, depending upon individual attributes of the pathological material studied.

As in previous work (1), the staining reaction for 19S human γ-globulin was specifically inhibited with unlabeled rabbit antibody against 19S human γ-globulin.

Other Methods.—Fluorescence microscopy and photography (7) and correlative histopathologic studies (1) of the customary variety were carried out, as described in previous publications.

RESULTS

Synovial Membranes in Rheumatoid Arthritis.—The cells which contain rheumatoid factor detectable with fluorescent immune complex comprise but a minute portion of the cell-rich inflammatory exudate that lies beneath the surface mesothelium of the thickened synovial membrane and in the stalks of the hypertrophic synovial villi. The cells with rheumatoid factor are plasma cells in various stages of development and include immature (Fig. 1), mature (Figs. 2 and 3), and Russell body types (Fig. 5).

While of similar appearance (Fig. 7), plasma cells which contain rheumatoid factor detectable with fluorescent aggregated human γ-globulin are more numerous (Fig. 9) than those which react with fluorescent immune complex. The lack of correspondence in the number of plasma cells which stain with the two reactants for rheumatoid factor is also shown by one of the blocking procedures. After exposure to unlabeled immune complex, the subsequent staining reaction with fluorescent aggregated human γ-globulin persists in a substantial population of plasma cells (Figs. 12 and 13). However prior treatment with unlabeled aggregated human γ-globulin completely inhibits the staining reaction with fluorescent immune complex.

Plasma cells containing rheumatoid factor have been demonstrated with fluorescent aggregated human γ-globulin, and in lesser number with fluorescent immune complex, in each example of active rheumatoid synovitis studied, whether occurring in adults (Figs. 1 to 3, 5, 7, and 9) or children, (Fig. 11), and in the presence or the absence of positive latex fixation tests. On one occasion, a needle biopsy provided sufficient synovial tissue for the detection of cellular rheumatoid factor.

Plasma cells which contain macroglobulin (19S human γ-globulin) detectable with fluorescent antibody are present in greater number than those containing rheumatoid factor, although the cellular type (Figs. 14, 15, and 19) and distribution are similar. While rheumatoid factor and macroglobulin are almost

exclusively formed and contained in the cytoplasm, these proteins are also demonstrable in the nucleus of an occasional plasma cell (Fig. 22).

Lymph Nodes in Rheumatoid Arthritis.—Two categories of cells in lymph nodes contain rheumatoid factor detectable with fluorescent immune complex: (*a*) germinal center cells, (Fig. 6) and plasma cells of immature, mature, and Russell body types (Fig. 4). While similar in appearance, these cellular sites of rheumatoid factor are less numerous than those demonstrated with fluorescent aggregated human γ-globulin (Figs. 8 and 10), and comprise but a small portion of the plasma cells and the germinal centers which contain macroglobulin (Figs. 17 and 18). In active rheumatoid arthritis, lymph nodes sometimes become hyperplastic, and in that event germinal cells containing macroglobulin may be so numerous that two or three immunofluorescent centers are seen in the same low-power field (Fig. 18), and as many as 50 or more may be counted in a single section (with an area of about 1 cm.^{2}). When a companion section of the same block is stained with fluorescent aggregated human γ-globulin, only three or four immunofluorescent centers, or at most five or six, may occur in the entire section, and in the reaction with fluorescent immune complex only one or two, or perhaps none, may be seen. The number of immunofluorescent plasma cells, principally internodular in distribution, also display progressively decreasing frequencies when lymph node sections are stained with fluorescent antibody for macroglobulin, with fluorescent aggregate, and with fluorescent complex.

Control Tissues.—Normal and pathological synovial and capsular tissues, lymph nodes, and connective tissues obtained from individuals without rheumatoid arthritis are not stained with the fluorescent immune complex. The synovial tissues include those obtained in various forms of arthritis (traumatic, degenerative, tuberculous, infectious, and gouty arthritis), pigmented villonodular synovitis, synovial chondromatosis, Reiter's syndrome, and Boeck's sarcoid. The lymph nodes include those of normal and hyperplastic appearance, as well as pathological lymph nodes in an example each of Boeck's sarcoid and Waldenström's macroglobulinemia. This control material, with a single exception, is likewise not stained with fluorescent aggregated human γ-globulin. In an unusual example of Waldenström's macroglobulinemia (5) approximately 50 per cent of the mononuclear cells (principally immature plasma cells) in a lymph node are stained with fluorescent aggregated human γ-globulin (Fig. 16) and with fluorescent antibody for macroglobulin (Figs. 20 and 21), but not with fluorescent immune complex. A notable serological feature of this case, which is discussed fully elsewhere (5), was the presence of very strong precipitin reactivity with aggregated human γ-globulin, with an unusual pH optimum, and a negative sheep cell agglutination test.

Macroglobulin detectable with fluorescent antibody (but not with fluorescent reactants for rheumatoid factor) is present in a few plasma cells and germinal

center cells of normal lymph nodes and exeptionally in plasma cells of synovial inflammatory exudates in conditions unrelated to rheumatoid arthritis.

DISCUSSION

As shown by a variety of serological tests (2), the serum of a majority of patients with rheumatoid arthritis, and a minority of individuals with other diseases, contains an unusual category of macroglobulins (8–10), called rheumatoid factor. This factor has the capacity to agglutinate particulate bodies, such as erythrocytes and latex particles, which have been coated with a sensitizing globulin, or reactant. Two principal categories of reactants are used in the serological tests for rheumatoid factor: rabbit immune (antibody-antigen) complex in the extensively studied sheep cell agglutination test (11) and altered human γ-globulin, or aggregated human γ-globulin (12), as in the precipitin test (13). In this and the previous study (1), two analogous categories of fluorescent reactants (fluorescent immune complex and fluorescent aggregate) were prepared, and rheumatoid factor was detected *in situ* in sections of tissues.

The synovial membranes and the lymph nodes of adults and children with rheumatoid arthritis and with positive, equivocal, and negative latex fixation tests contained rheumatoid factor. The cellular sites of origin of rheumatoid factor were found to be similar in kind (plasma cells and germinal center cells) when detected with the two categories of fluorescent reactants, but the number of cells stained with fluorescent aggregate was substantially greater than that stained with fluorescent immune complex. Prior treatment of sections with unlabeled aggregated human γ-globulin inhibited subsequent staining reactions with fluorescent aggregate and with fluorescent immune complex; whereas prior treatment with unlabeled immune complex diminished, but did not abolish, the staining reaction with fluorescent aggregate. These experimental findings are consistent with the behavior anticipated for cellular rheumatoid factor, were it primarily an antibody directed to an altered human γ-globulin and cross-reacting with rabbit γ-globulin, a possible interpretation that has been proffered from a consideration of the quantitative serological data obtained with the two reactants (14). However, an alternative explanation is possible; namely, that there are several (two or more) cellular rheumatoid factors directed against different antigenic components of aggregated human γ-globulin and that some of these components are present also in rabbit immune complex. In support of this possibility it has been shown that many sera from patients with rheumatoid arthritis produce two distinct precipitin lines with aggregated human γ-globulin when tested by gel diffusion (15). Moreover rheumatoid factor has been fractionated into distinct components capable of reacting with either rabbit antibody or altered human γ-globulin (16). Consequently it is possible that cellular rheumatoid factor of one type (and origin) reacts with a component present only in aggregated human γ-

globulin and that cellular rheumatoid factor of another type reacts with a component common to both the aggregated human γ-globulin and the rabbit immune complex. Further studies will be required for proof or denial of one or another of these explanations.

What are the pathogenic implications of these observations dealing with the cellular origin of rheumatoid factor? The studies show that cells which form the factor are an integral part of the synovial inflammatory exudate in rheumatoid arthritis and occur also in remote sites as well; namely, in the lymph nodes. The cells which form rheumatoid factor belong to the same families of cells which produce antibodies (17), 7S γ-globulin (18), and macroglobulin (1, 19, 5). Thus the cellular origin, as well as the chemical and the immunological attributes, of rheumatoid factor suggests an antibody-like nature and function. In that event a search should be undertaken in the tissues for the provocative antigen, such as an altered human γ-globulin (20), and for lesion-associated protein precipitates having the composition and attributes anticipated for a rheumatoid factor-antigen complex (21). This latter we have demonstrated recently in the kidney and spleen of a patient who died with amyloidosis secondary to rheumatoid arthritis. Macroglobulin, in part showing the reactivity of rheumatoid factor, and 7S γ-globulin were localized in the amyloid lesions, Figs. 23 and 24.

On the other hand, in rheumatoid arthritis as in certain other conditions plasma cells may proliferate and produce unusual proteins which do not have antibody function. The plasma cells and the abnormal serum proteins which occur in multiple myeloma and in Waldenström's macroglobulinemia are illustrative examples. None the less, a close relationship of rheumatoid factor to a pathogenic mechanism in rheumatoid arthritis seems evident from the characteristic presence in the actively inflamed synovial membrane of plasma cells forming and liberating rheumatoid factor.

SUMMARY AND CONCLUSIONS

In analogy with the two categories of reactants which are used in the serological tests for the unusual category of macroglobulins called rheumatoid factor, two fluorescent reactants have been prepared for the detection of rheumatoid factor *in situ* in tissue sections: fluorescent antigen–rabbit antibody (immune) complex, in the present study, and fluorescent aggregated human γ-globulin, in previous work.

Plasma cells in the synovial membrane and germinal center cells and internodular plasma cells in lymph nodes are the sites of origin of rheumatoid factor in active rheumatoid arthritis, whether occurring in adults or children. Plasma cells and germinal center cells which form rheumatoid factor detectable with fluorescent immune complex are less numerous than those which contain

factor demonstrable with fluorescent aggregate. In the same tissues, plasma cells and germinal center cells which contain macroglobulin (19S human γ-globulin) detectable with fluorescent antibody—but not showing the reactivity of rheumatoid factor—are more abundant than those containing rheumatoid factor.

While macroglobulin and rheumatoid factor are almost exclusively formed in the cytoplasm, these proteins are also detectable in the nucleus of an occasional plasma cell.

Normal and pathological synovial and capsular tissues, lymph nodes, and connective tissues obtained from individuals without rheumatoid arthritis are not stained with fluorescent immune complex or, except for an unusual example of Waldenström's macroglobulinemia, with fluorescent aggregate.

The cellular origin, as well as certain chemical and immunological attributes, of rheumatoid factor suggests an antibody-like nature and function. The observations cited are consistent with the behavior anticipated for cellular rheumatoid factor, were it primarily an antibody direct to an altered human γ-globulin and cross-reacting with rabbit γ-globulin. However, it is also possible that there are two or more cellular rheumatoid factors.

Lesion-associated protein precipitates having the composition anticipated for rheumatoid factor–antigen complex are localized in the amyloid depositions in kidney and spleen of an individual who died with amyloidosis secondary to rheumatoid arthritis.

This work was supported in part by a research grant from the United States Public Health Service and by the Bicknell Foundation.

We acknowledge our appreciation to Dr. Lee Ramsay Straub, Dr. Richard H. Freyberg, Dr. Philip D. Wilson, Jr., Dr. Ralph Heimer, Dr. Josue Corcos, and other members of the professional staff of the hospital for their cooperation during the course of this study and extend our thanks to Mr. Alfred Stoholski for his valuable assistance.

Addendum.—Since this paper was submitted, we have succeeded in preparing two fluorescent reactants for rheumatoid factor in contrasting colors: fluorescein-labeled immune complex (yellow-green) and rhodamine-labeled aggregated human γ-globulin (orange-red). When a section of lymph node in active rheumatoid arthritis is stained with these fluorescent reactants in mixture or in sequence, some of the germinal centers are orange-red and others, representing a smaller number, are green. The cellular origin of two rheumatoid factors has thus been demonstrated. Some of the plasma cells in a section of synovial membrane in active rheumatoid arthritis are stained orange-red and others, again in smaller number, are green. A few plasma cells appear to have combinations of orange-red and green colors, but this is stated only as a preliminary observation which requires further study.

BIBLIOGRAPHY

1. Mellors, R. C., Heimer, R., Corcos, J., and Korngold, L., Cellular origin of of rheumatoid factor, *J. Exp. Med.*, 1959, **110**, 875.

2. Ziff, M., The agglutination reaction in rheumatoid arthritis, *J. Chronic Dis.*, 1957, **5,** 644.
3. Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., and Metcalf, T. G., Isothiocyanate compounds as fluorescent labeling agents for immune serum, *Am. J. Path.*, 1958, **34,** 1081.
4. Edelman, G. M., Kunkel, H. G., and Franklin, E. C., Interaction of the rheumatoid factor with antigen-antibody complexes and aggregated gamma globulin, *J. Exp. Med.*, 1958, **108,** 105.
5. Kritzman, J., Kunkel, H. G., McCarthy, J., and Mellors, R. C., Studies of a Waldenström type macroglobulin with rheumatoid factor properties, data in preparation.
6. Lacy, P. E., and Davies, J., Demonstration of insulin in mammalian pancreas by the fluorescent antibody method, *Stain Technol.*, 1959, **34,** 85.
7. Mellors, R. C., and Munroe, J. S., Cellular localization of Rous sarcoma virus as studied with fluorescent antibody, *J. Exp. Med.*, 1960, **112,** 963.
8. Franklin, E. C., Holman, H. R., Müller-Eberhard, H. J., and Kunkel, H. G., An unusual protein component of high molecular weight in the serum of certain patients with rheumatoid arthritis, *J. Exp. Med.*, 1957, **105,** 425.
9. Heimer, R., Federico, O. M., and Freyberg, R. H., Purification of a rheumatoid factor, *Proc. Soc. Exp. Biol. and Med.*, 1958, **90,** 381.
10. Kunkel, H. G., Franklin, E. C., and Müller-Eberhard, H. J., Studies of the isolation and characterization of the "rheumatoid factor", *J. Clin. Inv.*, 1959, **38,** 424.
11. Rose, H. M., Ragan, C., Pearce, E., and Lipman, M. O., Differential agglutination of normal and sensitized sheep erythrocytes by sera of patients with rheumatoid arthritis, *Proc. Soc. Exp. Biol. and Med.*, 1948, **68,** 1.
12. Christian, C. L., Characterization of the "reactant" (gamma globulin factor) in the F II precipitin reaction and the F II tanned sheep cell aglutination test, *J. Exp. Med.*, 1958, **108,** 139.
13. Epstein, W., Johnson, A. M., and Ragan, C., Observations of a precipitin reaction between serum of patients with rheumatoid arthritis and a preparation of human γ-globulin, *Proc. Soc. Exp. Biol. and Med.*, 1956, **91,** 235.
14. Vaughan, J. H., Ellis, P. J., and Marshall, H., Quantitative considerations of the rheumatoid factor, *J. Immunol.*, 1958, **81,** 261.
15. Franklin, E. C., The precipitin reaction between rheumatoid factors and gamma globulin: studies by double diffusion in agar, *Arthritis and Rheumatism*, 1960, **3,** 16.
16. Heimer, R., Schwartz, E., and Freyberg, R. H., The different rheumatoid factors in the serum of an individual with rheumatoid arthritis, *J. Lab. and Clin. Med.*, in press.
17. Coons, A. H., Leduc, E. H., and Connolly, J. M., Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit, *J. Exp. Med.*, 1955, **102,** 49.
18. Ortega, L. G., and Mellors, R. C., Cellular sites of formation of gamma globulin, *J. Exp. Med.*, 1957, **106,** 627.

19. Dutcher, T. F., and Fahey, J. L., Immunocytochemical demonstration of intranuclear localization of 18S gamma macroglobulin in macroglobulinemia of Waldenström, *Proc. Soc. Exp. Biol. and Med.*, 1960, **103,** 452.
20. Kaplan, M. H., Current comment: the fluorescent antibody technic as a research tool in the study of connective tissue diseases, *Arthritis and Rheumatism*, 1959, **2,** 568.
21. Kunkel, H. G., Significance of the rheumatoid factor, *Arthritis and Rheumatism*, 1958, **1,** 381.

EXPLANATION OF PLATES

These are fluorescence photomicrographs of frozen sections, and descriptions pertain to the immunospecific fluorescence imparted by fluorescein-labeled reactants and antibodies.

PLATE 49

FIG. 1. Synovial membrane in rheumatoid arthritis. An immature plasma cell with eccentric nucleus devoid of staining and homogeneous cytoplasm containing rheumatoid factor (white areas). Stained with fluorescent rabbit immune complex. × 1500.

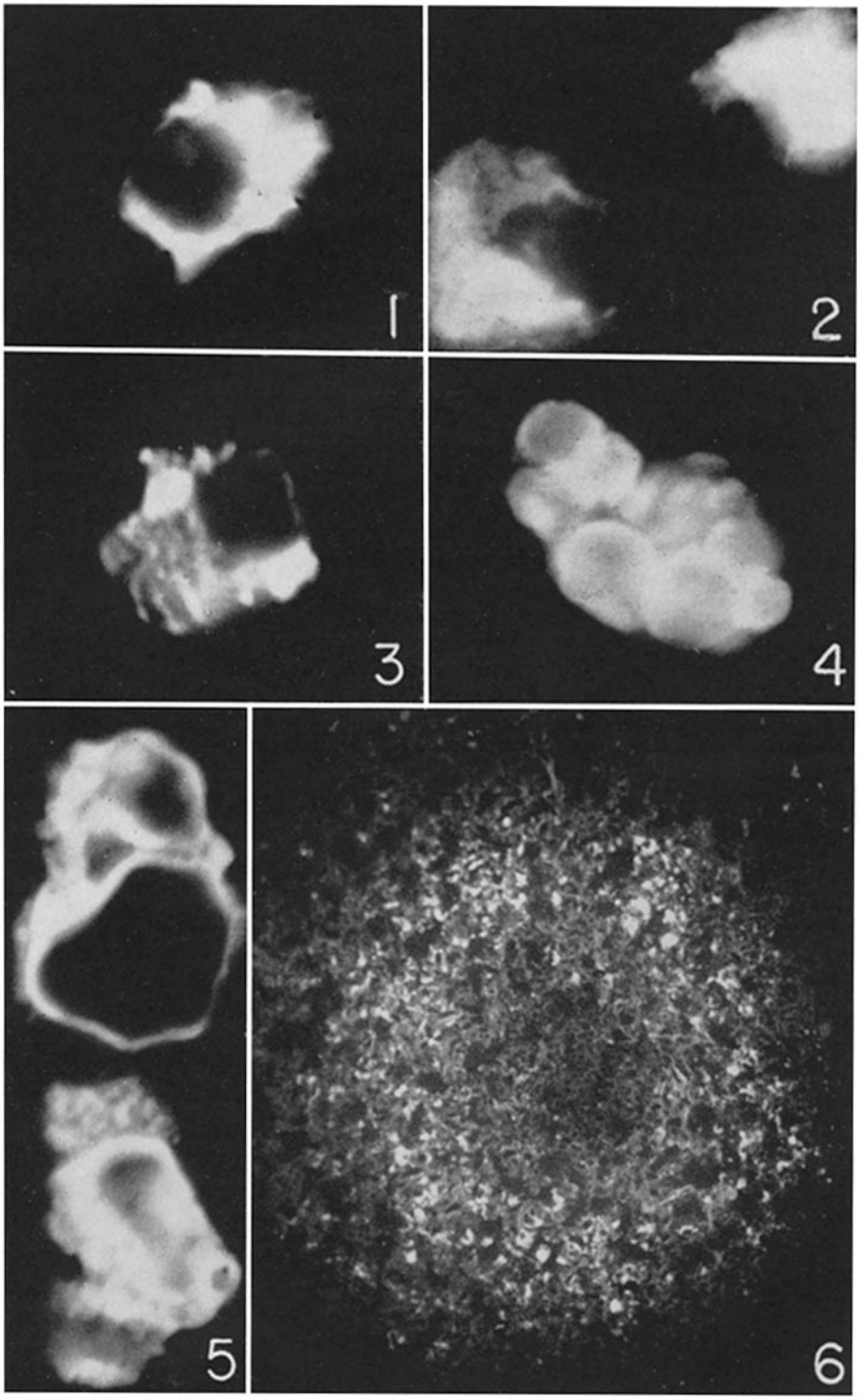
FIG. 2. Synovial membrane in rheumatoid arthritis. Two mature plasma cells containing rheumatoid factor in the cytoplasm. Fluorescent immune complex. × 1500.

FIG. 3. Synovial membrane membrane in rheumatoid arthritis. Mature plasma cell containing cytoplasmic rheumatoid factor with clumped and fibrillated distribution. Fluorescent immune complex. × 1500.

FIG. 4. Lymph node in rheumatoid arthritis. Russell body plasma cell containing rheumatoid factor in the cytoplasmic spherical bodies. Fluorescent immune complex. × 1500.

FIG. 5. Synovial membrane in rheumatoid arthritis. On the top, a Russell body plasma cell with bizarre appearance brought about by the confluence of the cytoplasmic bodies and loss of contents. Rheumatoid factor persists in outer membranes. On the bottom, tissue deposition of rheumatoid factor derived from cell residues. Fluorescent immune complex. × 1500.

FIG. 6. Lymph node in rheumatoid arthritis. Rheumatoid factor in germinal center cells occupying the outer half of the germinal center. More commonly, the central portion of the nodule is richest in content of rheumatoid factor, as shown in Fig. 10. Fluorescent immune complex. × 170.



(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)

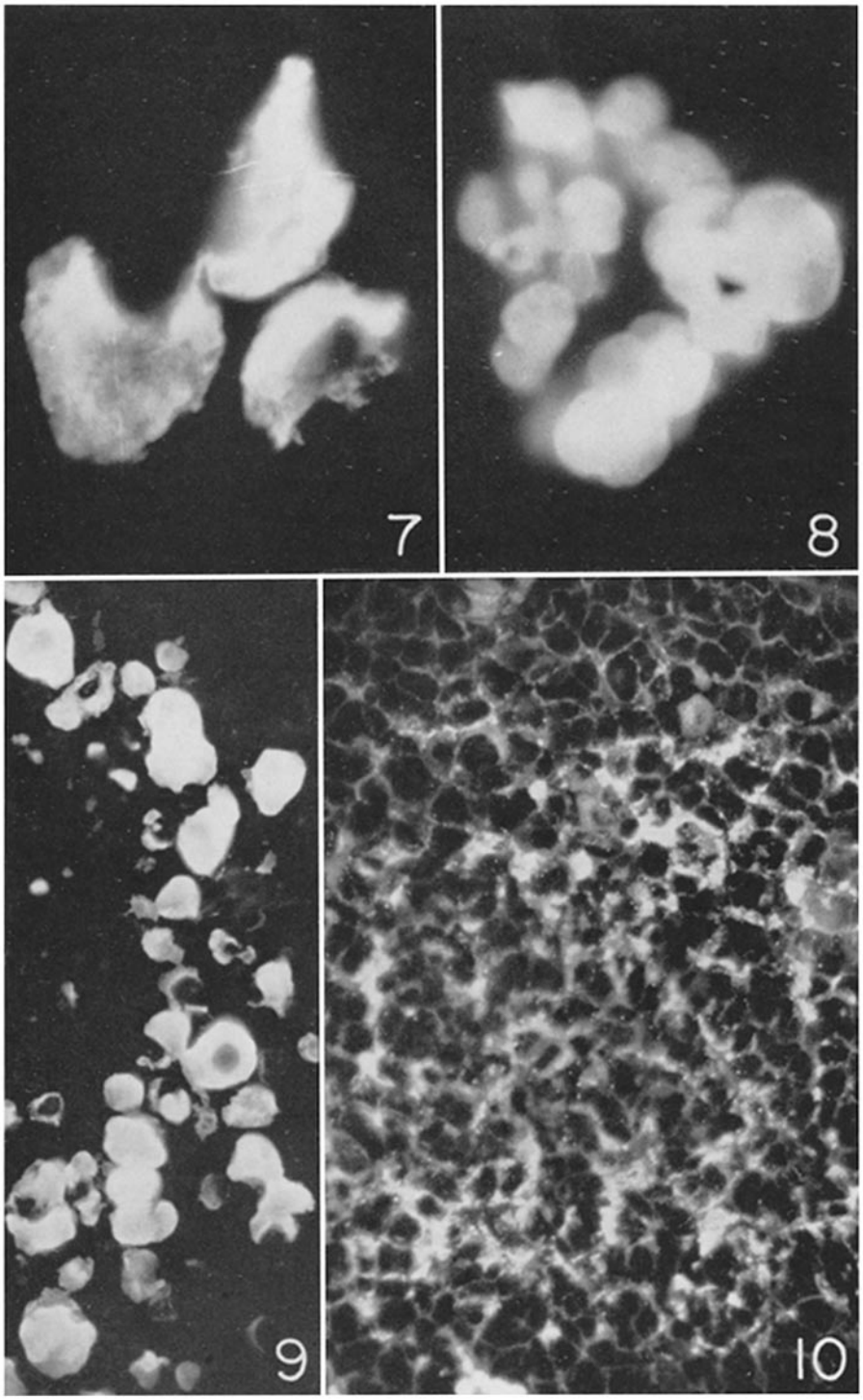
PLATE 50

FIG. 7. Synovial membrane in rheumatoid arthritis. Three mature plasma cells with cytoplasmic rheumatoid factor. Fluorescent aggregated human γ-globulin. $\times$ 1500.

FIG. 8. Lymph node in rheumatoid arthritis. Russell body plasma cell containing rheumatoid factor in the cytoplasmic spherical bodies. Compare with Fig. 4. Fluorescent aggregate. $\times$ 2000.

FIG. 9. Synovial membrane in rheumatoid arthritis. Rheumatoid factor in plasma cells of immature, mature, and Russell body types (identified at higher magnification), as well as in so called "prismatic" forms. Fluorescent aggregate. $\times$ 400.

FIG. 10. Lymph node in rheumatoid arthritis. Rheumatoid factor in the cytoplasm and cytoplasmic continua of germinal center cells (so called "large pale" cells). The surrounding mantle of mature lymphocytes (not shown) is always devoid of rheumatoid factor. See also Fig. 17. Fluorescent aggregate. $\times$ 510.



(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)

PLATE 51

FIG. 11. Synovial membrane in juvenile rheumatoid arthritis. A cluster of four or five mature plásma cells containing rheumatoid factor. Fluorescent aggregate. × 1500.

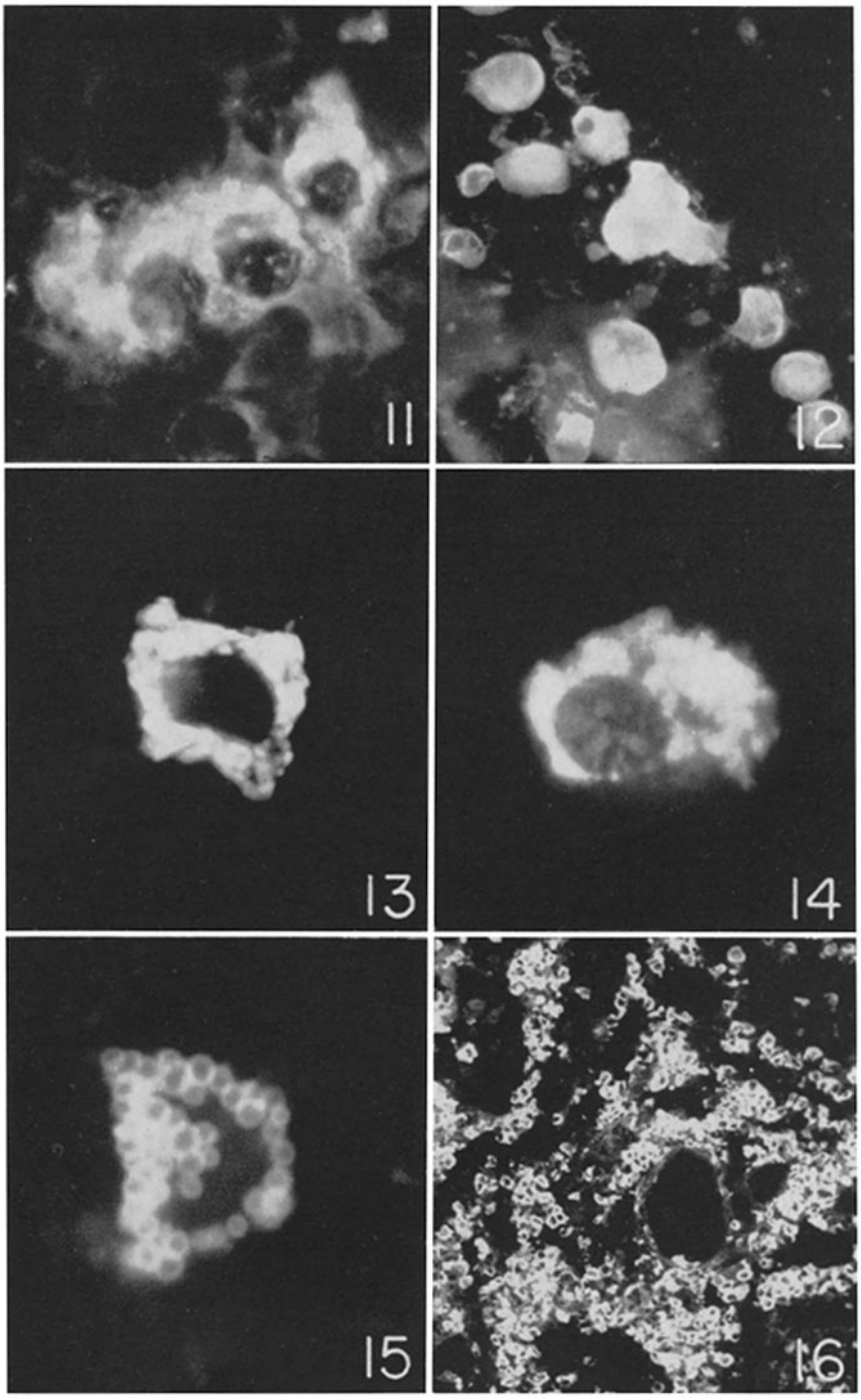
FIG. 12. Synovial membrane in rheumatoid arthritis. Field of plasma cells, similar to those in Fig. 9, containing rheumatoid factor. The section was first treated with unlabeled immune complex and then stained with fluorescent aggregate. × 400.

FIG. 13. Synovial membrane in rheumatoid arthritis. Plasma cell containing rheumatoid factor. Unlabeled immune complex followed by fluorescent aggregate. × 1500.

FIG. 14. Synovial membrane in juvenile rheumatoid arthritis. Plasma cell containing macroglobulin (19S γ-globulin) in the cytoplasm. See also Fig. 11. Fluorescent antibody stain. × 1500.

FIG. 15. Lymph node in juvenile rheumatoid arthritis. Russell body plasma cell containing macroglobulin in the cytoplasmic spherical bodies. Some of the plasma cells in this tissue were also shown to contain rheumatoid factor. Fluorescent antibody. × 1500.

FIG. 16. Lymph node in Waldenström's macroglobulinemia. Immature plasma cells and plasmablasts containing macroglobulin with the reactivity of rheumatoid factor. See also Figs. 20 and 21. Fluorescent aggregate. × 163.

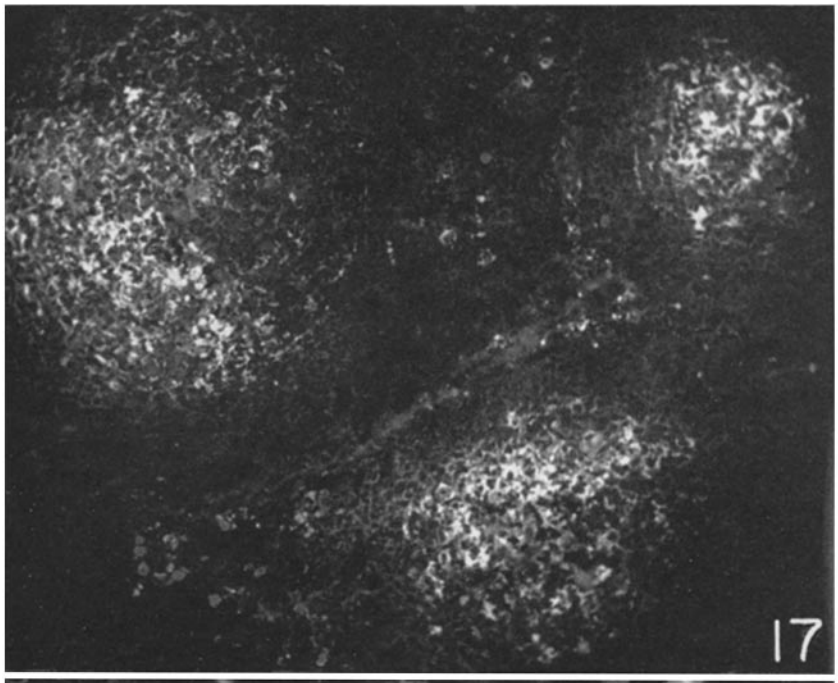


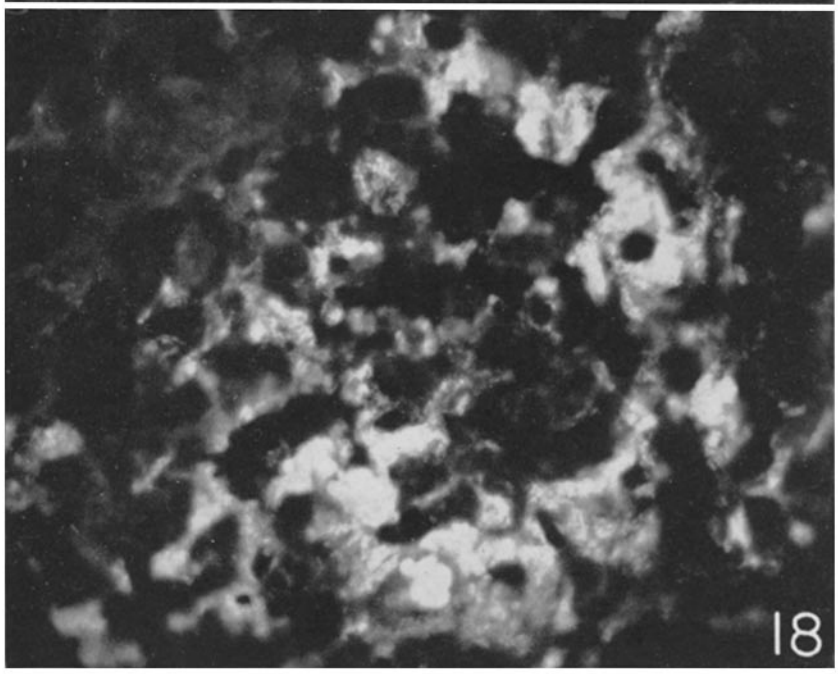
(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)

PLATE 52

FIG. 17. Lymph node in rheumatoid arthritis. Three germinal centers and scattered internodular plasma cells (identified at higher magnification) containing macroglobulin. Fluorescent antibody. × 136.

FIG. 18. Lymph node in rheumatoid arthritis. Germinal center containing macroglobulin. Fluorescent antibody. × 850.





(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)

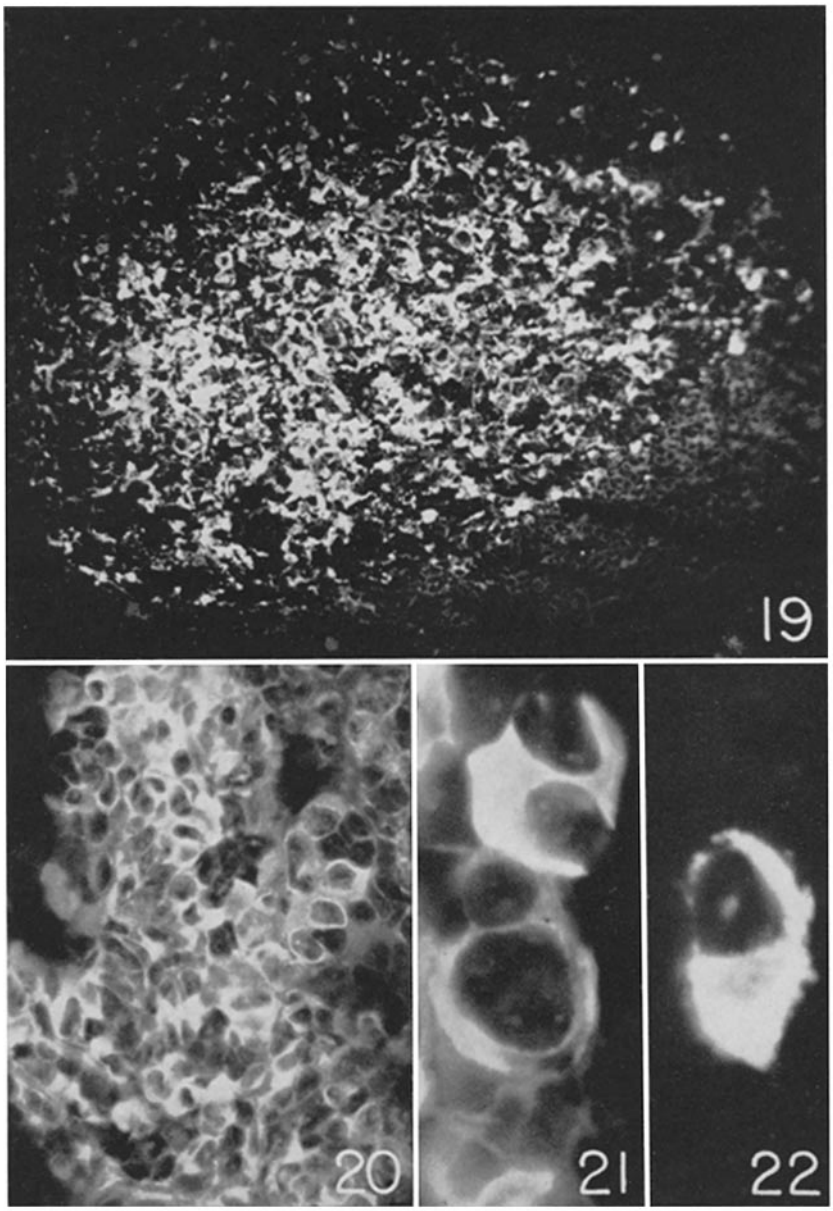
PLATE 53

FIG. 19. Lymph node in juvenile rheumatoid arthritis. Germinal center containing macroglobulin. Fluorescent antibody. × 200.

FIG. 20. Lymph node in Waldenström's macroglobulinemia. Immature plasma cells and plasmablasts containing macroglobulin. Fluorescent antibody. × 400.

FIG. 21. Lymph node in Waldenström's macroglobulinemia. Similar to Fig. 20. × 1000.

FIG. 22. Synovial membrane in rheumatoid arthritis. Mature plasma cell containing macroglobulin in cytoplasm and in a small central focus in the nucleus. Rheumatoid factor has also been detected in the nucleus on rare occasion. Fluorescent antibody. × 2000.

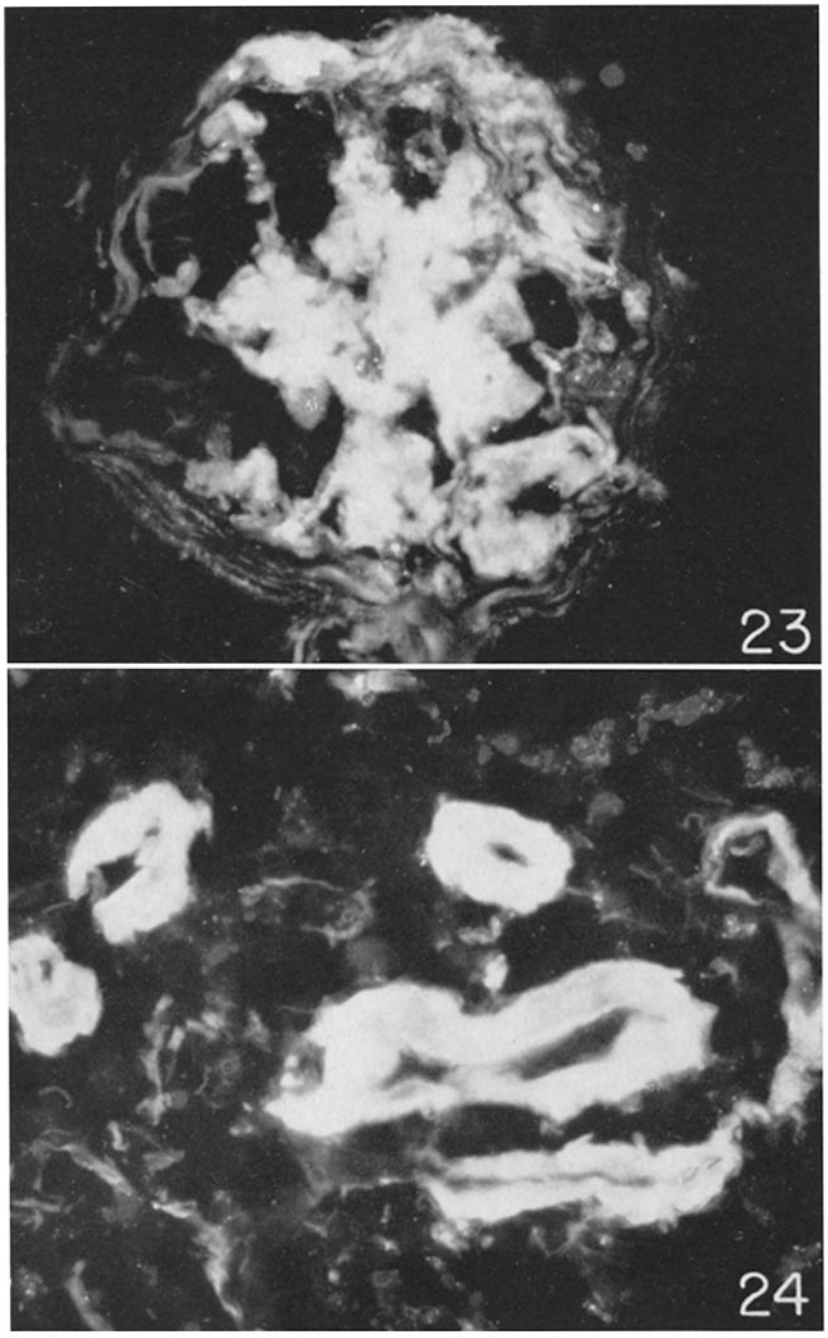


(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)

PLATE 54

FIG. 23. Kidney in amyloidosis secondary to rheumatoid arthritis. Rheumatoid factor in glomerular capillary tuft in locations corresponding to amyloid deposits. Macroglobulin lacking the reactivity of rheumatoid factor and 7S γ-globulin were also detectable in these sites. Fluorescent aggregate. $\times$ 400.

FIG. 24. Spleen in amyloidosis secondary to rheumatoid arthritis. Rheumatoid factor in thickened walls of small blood vessels, in locations corresponding to amyloid deposits. Macroglobulin lacking the reactivity of rheumatoid factor and 7S γ-globulin were also detectable in these sites. Fluorescent aggregate. $\times$ 510.



(Mellors *et al.*: Pathogenesis of rheumatoid arthritis)