CELLULAR ORIGIN OF RHEUMATOID FACTOR

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The rheumatoid factor, a group of unusual macroglobulins, is demonstrable in the serum of the majority of patients with rheumatoid arthritis and in a relatively small proportion of patients with other diseases (1). The presence of this factor can be demonstrated by its reaction with human γ-globulin (Cohn fraction II) in such serological procedures as the tanned sheep cell agglutination (2) and latex fixation tests (3), as well as by precipitin tests (4). The reactive component of γ-globulin seems to be an aggregate (5) with high sedimentation coefficients, which forms an insoluble complex with the rheumatoid factor. The origin and site of production of the rheumatoid factor has been unknown. In the present study, fluorescein-labelled aggregated human γ-globulin was used as a specific reagent for its detection *in situ*. The results show that the factor is present in certain cells of the synovial tissues, lymph nodes, and subcutaneous nodules in active rheumatoid arthritis.

Materials and Methods

Fluorescein-Labelled Aggregated Human γ-Globulin.—Human γ-globulin (Cohn fraction II, E. R. Squibb Lot 1788), obtained as a gift from the American National Red Cross, was conjugated at a concentration of 20 mg. protein per ml. with fluorescein isothiocyanate (Sylvana Chemical Co., Orange, New Jersey), in accordance with the procedure of Riggs and associates (6), and dialyzed against buffered saline (0.15 M sodium chloride containing 0.01 M phosphate, pH 7.0) until free of unconjugated fluorescein. The fluorescein-labelled human γ-globulin preparation was heated in a water bath to 62°C. for 10 minutes, and 40 per cent by volume of 2.18 M Na_2SO_4 was then added, with stirring, in a manner used by Christian (5). A precipitate was collected after overnight storage in the cold, dissolved in saline, and dialyzed against phosphate buffer (0.15 M, pH 7.0). The dialyzed preparation was centrifuged at 18,400 R.P.M. for 1 hour prior to use. This preparation will be referred to in this report as fluorescein-labelled aggregated human γ-globulin. This reagent was six times more reactive in precipitation reactions with rheumatoid serums than a similarly prepared but unheated γ-globulin preparation, and furthermore was five times more reactive than a heated preparation in which the Na_2SO_4 treatment was omitted. The various preparations were also analyzed by ultracentrifugation. Fluorescein-labelled aggregated human γ-globulin contained approximately 34 per cent of the 7S component and 66 per cent of an aggregate having sedimentation coefficients varying from approximately 10S to 70S. The analysis was performed in a Spinco,

model E ultracentrifuge, at 20°C., at 47,660 R.P.M., using a double sector cell No. 5578, filled with buffer in one compartment so as to facilitate accurate calculation of the concentration of the components. Little information is available concerning the stability of the reagent. Small amounts of material tend to precipitate out of solution at a slow rate, resulting in diminution of activity due to concentration changes. The over-all potency of such preparations however remains constant on storage in the cold over a period of a few weeks, when concentration effects are taken into account.

Other Reagents.—Two other fluors were tested for their reactivity with rheumatoid factor by means of precipitin tests as well as by staining of companion tissue sections. Fluorescein-labelled human γ-globulin (Cohn fraction II) was tested directly. This fluor reacted only moderately in precipitin type tests, and furthermore provided only a barely perceptible, but yet apparently specific, apple-green staining pattern in tissue sections containing rheumatoid factor. The other fluor was that portion of the heat-treated fluorescein-labelled γ-globulin which failed to be precipitated by the Na_2SO_4 method. This reagent was a poor precipitating agent for rheumatoid factor, and failed to give apple-green staining with appropriate tissue sections.

Fluorescent Antibody against Human γ-Globulins.—The starting material in the preparation of fluorescent antibody for 19S human γ-globulin was a mixture of antiserums prepared in rabbits by the injection of purified macroglobulins obtained from patients with macroglobulinemia. These antiserums cross-reacted with normal γ_1-macroglobulin. The antigens and antiserums have been described elsewhere (7). The antiserums and a γ-globulin fraction prepared by cold ethanol fractionation were absorbed with 7S γ_2-globulin and β-globulin (prepared by zone electrophoresis in starch) until the antibodies were specific for macroglobulin. A γ-globulin fraction was also conjugated with fluorescein isothiocyanate and then absorbed in the same manner as the unlabelled γ-globulin fraction. This fluor was used for the detection of human 19S γ-globulin.

The starting material in the preparation of fluorescent antibody for 7S and/or 19S γ-globulin was antiserum produced by immunizing a goat with human γ-globulin (Cohn fraction II). By the agar diffusion technique, precipitin lines of activity with 7S and 19S human γ-globulins, β_1- and β_2-globulins, and albumin were produced with this antiserum. The crude γ-globulin fraction of the goat antiserum (prepared by cold ethanol fractionation) was conjugated with fluorescein isothiocyanate at a protein concentration of 20 mg. per ml., dialyzed free of unconjugated fluorescein, frozen in small aliquots, and stored at −30°C. As needed the conjugate was absorbed with human serum albumin and β-globulin in order to obtain a fluor characterized by precipitin activity with 7S human γ-globulin but not with albumin and β-globulin. This immunologically characterized fluor was used for the detection of 7S and/or 19S human γ-globulin.

Source and Nature of Tissues.—Surgical material was obtained from 10 patients with active classical rheumatoid arthritis: 9 females and 1 male, ranging in age from 41 to 65 years, with a duration of disease from 1 to 34 years, having been treated with salicylates, gold and corticosteroids, with the exception of two individuals who did not receive steroids. The patients had positive latex fixation tests, with titers ranging from 1:2,560 to 1:152,000. The following soft tissue specimens were taken for the study: synovial tissues 8 (hand and wrist joint 7, knee joint 1); lymph nodes 11 (axillary, from 1 patient); subcutaneous nodules 4 (elbow region, from 2 patients); skin biopsy 2 (calf region, from 2 patients); and skeletal muscle biopsy 1. Tissue blocks, numbering 130 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 43, routine 52; lymph nodes, special 4, routine 12; subcutaneous nodules, special 6, routine 7; skin, special 2, routine 2; and muscle, special 1, routine 1. In addition 6 blocks of accompanying bone and articular cartilage were decalcified for paraffin embedding and sec-

tioning. The gross and the microscopic findings in the synovial tissues were in each case "typical" of rheumatoid synovitis; the subcutaneous nodules were sparsely cellular rheumatoid granulomas; the lymph nodes were hyperplastic; and the skin and muscle were normal.

Control surgical material from 22 patients, 13 to 72 years of age, 14 males and 8 females, without rheumatoid arthritis and with negative latex fixation tests for the rheumatoid factor provided the following tissue blocks for study: synovial tissue, special 30, routine 35; lymph nodes, special 10, routine 8; and various connective tissues, special 9, routine 11. The synovial tissues were normal and pathological, including in the latter category, chronic synovitis associated with degenerative and traumatic osteoarthritis, tuberculous synovitis, pigmented villonodular synovitis, and an example of chronic proliferative and exudative villous synovitis closely simulating rheumatoid synovitis in many of its microscopic features. In the category of connective tissues were normal loose and dense fibrous, elastic, fibrocartilaginous, and adipose tissues as well as gouty tophus, ganglion, chronic bursitis, herniated nucleus pulposus, acute and chronic inflammation, fibrinous exudation, fibrinoid, and hyaline and mucoid degeneration. The lymph nodes were variously normal, hypoplastic, and hyperplastic and some contained carbonaceous pigment or hemosiderin.

Autopsy material obtained from a male 40 years of age, without rheumatoid arthritis and with negative latex fixation test for the rheumatoid factor, provided a total of 35 frozen blocks and 35 paraffin blocks of the following normal organs for control study: heart, lungs, liver, spleen, kidneys, parotid glands, stomach, small and large intestines, pituitary, thyroid, adrenals, pancreas, testes, skeletal muscle, and brain.

Preparation of Frozen Sections.—Fresh tissue blocks with a thickness of about 5 mm. and an area of about 1 $cm.^2$ were placed on aluminum foil (strips of cardboard serving as supports), inserted into large mouth, flattened pyrex tubes, sealed with tightly fitting rubber stoppers, frozen at −70°C. for 5 minutes in a bath of dry ice and acetone, and stored in a freezer at −25° to −30°C. As needed, these blocks were transferred to a microtome cryostat at −25° to −30°C., mounted with ice on object holders, and sectioned at an indicated thickness of 4μ. The frozen sections were transferred to slides, rapidly thawed, dried at room temperature for about 10 minutes, and stored in the refrigerator in sealed containers until needed. The microtome was a Spencer model 820 lubricated with Hamilton Watch oil No. T-3358 (Hamilton Watch Co., Lancaster, Pennsylvania).

Fluorescence Staining Procedures.—As needed, frozen sections were removed from the refrigerator, air-dried, fixed with anhydrous acetone for 15 minutes at room temperature, air-dried, washed with buffered saline solution (4 changes each of 4 minutes), drained, blotted with absorbent paper, and treated with the appropriate absorbed fluorescein-labelled protein solution or other reactant. To remove the non-specific staining shown by the cytoplasmic granules of eosinophilic leukocytes, 0.5 to 1.0 ml. aliquots of the fluors were, as needed for 1 day's work, absorbed with acetone-precipitated, saline-insoluble, lyophilized rabbit bone marrow powder (8), in the amount of 150 mg. tissue powder per 1 ml. fluor, for 1 hour at room temperature. After absorption the fluorescein-labelled protein solutions were centrifuged at 18,400 R.P.M. for 1 hour at 5°C. The supernatant fluid was used for staining purposes and comprised 80 and 60 per cent, respectively, of the starting volumes (prior to centrifugation) of labelled aggregated γ-globulin and fluorescent antibodies.

The tissue sections were covered with a drop or two of fluor, the slides were covered with Petri dishes lined with saline-moistened filter paper, incubated at room temperature for a standard period (which after preliminary trials was set at 60 minutes), washed free of unbound fluor with several changes of buffered saline solution, washed and mounted in buffered glycerin, and sealed with coverslips for microscopic study. Fluorescein-labelled aggregated human γ-globulin was used, with the appropriate control procedures noted below, to detect rheumatoid factor: fluorescein-labelled goat antibody, appropriately absorbed and immuno-

logically characterized, to detect 7S and/or 19S human γ-globulin; and fluorescein-labelled rabbit antibody of known immunospecificity to detect 19S human γ-globulin.

Control Procedures.—The specificity of the apple-green staining pattern in tissue sections exposed to fluorescein-labelled aggregated human γ-globulin and attributed to the presence of the rheumatoid factor was confirmed by the fact that the staining reactions were completely or substantially, inhibited by prior treatment of serial sections either (*a*) with unlabelled aggregated human γ-globulin or (*b*) with unlabelled rabbit antibody against 19S human γ-globulin, (but not, for example, with unlabelled rabbit antibody against sheep erythrocytes). In the inhibition procedures, the sections were exposed to the appropriate protein solution for 1 hour at room temperature, followed by washing in buffered saline and staining with fluorescein-labelled aggregated human γ-globulin for 1 hour.

The specificity of the staining reaction for 19S human γ-globulin was confirmed by the fact that staining was inhibited by prior treatment of serial sections with unlabelled rabbit antibody against 19S human γ-globulin. In addition, prior treatment of serial sections with unlabelled aggregated human γ-globulin was used to inhibit staining of rheumatoid factor. In the inhibition procedures, the sections were exposed to the appropriate protein solution for 1 hour, followed by washing in buffered saline and staining with fluorescein-labelled rabbit antibody against 19S human γ-globulin for 1 hour.

The specificity of the staining for 7S and/or 19S human γ-globulin was confirmed by the fact that the staining was inhibited by absorption of the fluor with human γ_2-globulin so as to remove all detectable precipitin activity with this antigen. Tissue sections were exposed for 1 hour to the absorbed fluor.

Fluorescence Microscopy and Photography.—A Zeiss fluorescence microscope was used. This monocular instrument was fitted with an achromatic objective (10×), neofluar objectives (16×, 40×), apochromatic immersion objective (100×), and corrected eyepiece (12.5×), and illuminated with an Osram HBO-200 mercury arc. For visual work, which was carried out on all sections from lowest power to highest, the exciter filter was UG5 and the barrier filter was BG23. For color photography the exciter filter was UG2, the barrier filter was BG23, and the photographic emulsion super anscochrome, color, daylight, 120 mm. roll film. For photography in black and white the exciter filter was BG12, the barrier filter, OG4, and the photographic emulsion Ilford FP3, a fine grain panchromatic film. The camera reduced the magnification by a factor of approximately one-third. Exposure times varied between 1 and 4 minutes for photography in black and white and were approximately twice that time for color. In the present study approximately 300 frozen sections, treated with fluors, were examined and described and almost 400 individual photomicrographs were made as a permanent record of the observations.

Other Histological Procedures.—The formalin-fixed blocks of tissue were embedded in paraffin, cut at 4 μ, and stained with Harris' hematoxylin and picric-acid-eosin, hematoxylin-eosin and alcian Blue, and, when indicated, phosphotungstic acid–hematoxylin.

Frozen sections, companions to those treated with fluorescein-labelled proteins as well as those already studied by fluorescence microscopy, were fixed in a mixture of acetic acid (5 parts by volume), ethanol (85 parts), and 40 per cent formaldehyde (10 parts) and stained with hematoxylin and eosin. It was possible in many instances to study the same fields in both the fluorescence and the light microscopes in sequence. For example, in lymph nodes the proportion of germinal centers containing rheumatoid factor was determined by counting first the number of specific apple-green fluorescent centers in a section appropriately stained and then counting the total number of germinal centers in the same section after staining with hematoxylin and eosin.

Latex Fixation Test.—This test was carried out by the procedure described by Singer and Plotz (3). The latex particles of 0.83 micra diameter were a product of the Dow Chemical

Company, and the human γ-globulin (Cohn fraction II) was a gift of the American National Red Cross. Latex fixation tests were also performed in which fluorescein-labelled human γ-globulin was substituted for the standard γ-globulin component. These experiments demonstrated that introduction of the dye into the protein reactant did not affect the titer of seropositive and negative samples.

RESULTS

Synovial Membranes in Rheumatoid Arthritis.—The cells containing rheumatoid factor are seen in characteristic apple-green color after staining with fluorescein-labelled aggregated γ-globulin, in contrast with other, and generally more numerous, inflammatory cells (lymphocytes principally) seen in active rheumatoid synovitis and faintly discerned by the blue-violet color of the cytoplasm and by the dark nuclei, essentially devoid of color. The cells with rheumatoid factor present the following microscopic appearance, Figs. 1 to 12. The nucleus is large, oval or round, usually eccentric, and devoid of color or shows some apple-green "flare" imparted by the cytoplasm. The cytoplasm is brightly fluorescent in crisp apple-green color, and the nuclear and the cell boundaries are usually sharply delimited. The cells containing rheumatoid factor are usually discrete, occasionally binucleate, Fig. 5, rarely show sharing of cytoplasm by two adjacent cells, and constitute but a small minority of the mononuclear inflammatory cells present. Cells of many sizes and shapes contain rheumatoid factor in active rheumatoid synovitis but the majority, if not all, of these are plasma cells in various stages of development and maturity, characterized principally by the amount and the distribution of the cytoplasm. Some of these cells have but a narrow rim of apple-green cytoplasm distributed around a centrally located, dark colorless nucleus and sometimes extending into stellate-shaped cell processes. Such cells when discrete and in the company of manifestly mature plasma cells are interpreted as immature plasma cells, Fig. 1. Other cells containing rheumatoid factor have substantial amounts of bright apple-green cytoplasm, and the colorless nucleus is eccentrically displaced. These cells are various forms of mature plasma cells, Figs. 2 to 8, including the Marschalko types. The apple-green cytoplasm may be homogeneous, indistinctly or occasionally distinctly granular, finely vacuolated, or may show a juxtanuclear "empty space," Fig. 4, or rarely a fibrillar appearance. The cell boundaries are usually rounded and distinct, Figs. 2 and 3, but some mature plasma cells have polyhedral or irregular shapes with an eccentric colorless nucleus and apple-green cytoplasm that is vacuolated or thinned out along one margin or has other features suggesting secretory activity, Figs. 6 to 8.

Although infrequent in number, the Russell-body plasma cells, Figs. 9 to 12, are the most distinctive of the cells containing rheumatoid factor. Russell bodies in their earliest detectable form are seen as small, round and oval apple-green cytoplasmic globules, Fig. 9, of fairly uniform size. These cytoplasmic

bodies increase in size, displace the colorless nucleus to the side, Figs. 10 and 11, or to the center of the cell, Fig. 12, or otherwise obscure it, and present a characteristic appearance imparted by the apple-green fluorescence of their surfaces and visualized to best advantage in certain planes of focus as rings, Figs. 10 to 12 and 14. Each ring represents an equatorial plane of a Russell body. The coalescence of neighboring Russell bodies may occasionally be seen, Fig. 13. Very rarely, free Russell bodies, Fig. 14, are found in the interstitial spaces, and irregular shaped masses also containing rheumatoid factor and appearing like the remnants or the products of Russell body, or other mature, plasma cells are observed, Fig. 15.

When sections in active rheumatoid synovitis are treated with fluorescein-labelled rabbit antibody against 19S human γ-globulin, the cytoplasm of plasma cells with types, numbers, and distributions similar to those containing rheumatoid factor is seen in characteristic apple-green color, Fig. 16. Since this staining was inhibited by prior treatment of sections with unlabelled aggregated human γ-globulin, it is apparent that the 19S γ-globulin is rheumatoid factor. Plasma cells are also seen which contain 7S and/or 19S γ-globulin or lack detectable γ-globulin.

Rheumatoid factor-containing plasma cells conforming to the types illustrated exist in active rheumatoid synovitis in varying numbers in the cell-rich inflammatory exudate that forms beneath the surface mesothelium of the thickened synovial membrane, in the stalks of the hypertrophic synovial villi, Fig. 17, and in clusters about small blood vessels. Such apple-green plasma cells are rarely seen also in the interstices of the capsular fibrous tissue. The distribution of rheumatoid factor-containing cells corresponds in general to that seen for plasma cells in companion hematoxylin-eosin sections but numerically constitutes only a minority of the plasma cells that are present. Lymphoid cells, including those occurring in aggregates or so called lymphoid foci (in the present material all were devoid of germinal centers), do not contain rheumatoid factor nor do any of the following cells or structures in the rheumatoid synovial tissues examined. These include mesothelial cells, all varieties of inflammatory cells (among them lymphocytes, neutrophiles, eosinophiles, macrophages), erythrocytes, fibroblasts, vascular endothelium, reticulum cells, collagen, elastic, and reticulin fibers, fibrocartilage, ground substance, connective tissue mucoid, fibrinoid, hyaline, and fibrinous and serous exudate.

Lymph Nodes in Rheumatoid Arthritis.—As shown by their characteristic apple-green color when stained with fluorescein-labelled aggregated γ-globulin and in contrast with the blue-violet and blue-white appearance of other structures, rheumatoid factor-containing cells in lymph nodes in active rheumatoid arthritis comprise two categories: (*a*) germinal-center cells, Figs. 18 to 21, of lymphoid nodules and (*b*) plasma cells, Fig. 22, of various immature and mature types. Approximately one in ten germinal centers (in the hyperplastic

lymph nodes obtained from one case of active rheumatoid arthritis) contained rheumatoid factor. The intrinsic cells (9) of these germinal centers have large, solitary colorless nuclei; narrow strips or wider areas of apple-green cytoplasm with homogeneous, or finely or coarsely granular, appearance; and stellate-shaped, cell processes which touch and merge with those of adjacent cells and form a cytoplasmic continuum of apple-green color, distributed throughout the germinal center, with areas devoid of color (some of these marking the imprint of extrinsic cells), and tapering off or ending abruptly as the surrounding mantle of mature lymphocytes is reached, Figs. 18 to 20. The size of the rheumatoid factor–containing germinal centers varies as does also the intensity of apple-green fluorescence, the latter being in some instances at the highest level encountered in any tissues in the present study. The plasma cells with rheumatoid factor are usually located in the internodular medullary cords and occasionally superimposed on the centers or on their mantles of mature lymphocytes (themselves devoid of rheumatoid factor) or occurring elsewhere.

When serial sections of lymph nodes in active rheumatoid arthritis are treated with fluorescein-labelled rabbit antibody against 19S human γ-globulin, the apple-green staining pattern (of germinal centers and internodular plasma cells) is comparable to, and by inhibition procedures shown to be identical with, that obtained for rheumatoid factor. When serial sections are treated with fluorescein-labelled goat antibody against 7S and/or 19S human γ-globulin, approximately eight out of ten germinal centers are found to contain 7S and/or 19S γ-globulin (whereas, either rheumatoid factor or 19S γ-globulin is demonstrable in but one in ten germinal centers). Plasma cells, of various immature and mature types, containing 7S and/or 19S γ-globulin are morphologically comparable to those containing rheumatoid factor and are present in the internodular medullary cords, Figs. 22 and 23, and elsewhere.

Subcutaneous Nodules in Rheumatoid Arthritis.—Apple-green plasma cells containing rheumatoid factor are occasionally seen in subcutaneous nodules in rheumatoid arthritis and in the present study occur, not in the central necrotic zone or the intermediate palisading, cellular zone but in paravascular foci and elsewhere in the outer zone of fibrous and inflammatory tissue, Fig. 24.

Control Tissues.—When sections of normal or pathological synovial and capsular tissues, lymph nodes, and connective tissues, and various normal parenchymatous organs are obtained from individuals without rheumatoid arthritis and treated with fluorescein-labelled aggregated human γ-globulin no apple-green staining for rheumatoid factor is seen. The synovial tissues studied include typical examples of chronic synovitis associated with degenerative and traumatic osteoarthritis, tuberculous synovitis, pigmented villonodular synovitis, and chronic proliferative and exudative villous synovitis with prominent lymphoid foci and other microscopic features closely simulating rheumatoid synovitis. The connective tissues comprise normal structures

(loose and dense fibrous, elastic, fibrocartilaginous, and adipose tissues), pathological depositions (mucoid, hyaline, fibrinoid, and fibrin), and acute and chronic inflammatory exudate, as well as gouty tophus, ganglion, chronic bursitis, and herniated nucleus pulposus. The normal organs studied include heart, lungs, liver, spleen, lymph nodes, kidneys, parotids, stomach, small and large intestines, pituitary, thyroid, adrenals, pancreas, testes, skeletal muscle, and brain.

Biopsies of normal skin and skeletal muscle from patients with rheumatoid arthritis fail to stain with fluorescein-labelled aggregated γ-globulin.

DISCUSSION

Evidence has been presented by others (5, 10) that in the various serological reactions for rheumatoid factor using human γ-globulin (Cohn fraction II), the reactant is an aggregated γ-globulin (with high sedimentation constant) which forms an insoluble complex (precipitate) with the rheumatoid factor. According to current opinion this reaction does not fulfill all of the criteria of an antigen-antibody system. When coupled with fluorescein the reactant maintained its chemical reactivity with rheumatoid factor. A purpose of this investigation has been to show that fluorescein-labelled aggregated human γ-globulin applied to frozen sections provides a specific and sensitive stain for the microscopic demonstration of cells containing rheumatoid factor in the synovial tissues, the lymph nodes, and the subcutaneous nodules in individuals with active, classical rheumatoid arthritis and strongly positive latex fixation tests for rheumatoid factor. When studied in paraffin sections stained with hematoxylin and eosin, the microscopic features observed in the synovial membranes fulfilled the histological criteria for rheumatoid arthritis as outlined by a committee of the American Rheumatism Association (11) and comprising three or more of the following: "marked villous hypertrophy; proliferation of superficial synovial cells, often with palisading; marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form *lymphoid nodules*; deposition of compact fibrin, either on surface or interstitially; and foci of cell necrosis." Of these features, cell necrosis was not a prominent occurrence in the present material, and the so called lymphoid nodules were designated simply as lymphoid aggregates or foci for, while spherical, they did not possess true germinal centers. This, however, is the usual appearance of lymphoid (round-cell) aggregates in rheumatoid synovitis (as may be seen also in other forms of chronic exudative synovitis) because only exceptionally do they contain germinal centers corresponding to those seen in lymph nodes.

Cells containing rheumatoid factor were demonstrated in multiple sections of each example of active rheumatoid synovitis studied, although their numbers varied greatly from field to field and from specimen to specimen. These cells

were plasma cells of various immature and mature types diffusely distributed principally in the submesothelial inflammatory exudate, and the rheumatoid factor was localized in the cytoplasm or rarely was seen in extracellular sites, apparently derived from plasma cellular residues or "secretions." The lymphoid aggregates or mature lymphocytes elsewhere did not contain rheumatoid factor, nor did mesothelial cells, or other normal cells, inflammatory cells, normal structures, or pathological depositions. Plasma cells containing rheumatoid factor were sometimes seen close to the periphery of lymphoid aggregates. By utilizing the fluorescent antibody method, plasma cells containing 19S human γ-globulin were demonstrated in active rheumatoid synovitis. The 19S γ-globulin was probably entirely rheumatoid factor. Of all the plasma cells present (their abundance being judged in hematoxylin-eosin sections), many, and sometimes apparently the majority, did not contain detectable γ-globulin of any type (rheumatoid factor, 19S, or 7S).

The presence of cells containing rheumatoid factor and comprising two categories (germinal-center cells and plasma cells) was confirmed in multiple sections of each of several hyperplastic lymph nodes obtained from a patient with active rheumatoid arthritis. Approximately one in ten germinal centers contained rheumatoid factor which was localized in the cytoplasm of germinal-center cells and in their characteristic protoplasmic processes and continua (some of which may actually be extracellular depositions). The surrounding mantle of mature lymphocytes about the germinal centers of lymphoid nodules (follicles) were devoid of rheumatoid factor, as were all other remaining cells except plasma cells which were present in small numbers in the internodular medullary cords and occasionally elsewhere. In one out of ten germinal centers and in some plasma cells 19S γ-globulin was demonstrated and was principally, if not entirely, rheumatoid factor. On the other hand, approximately eight out of ten germinal centers were found to contain 7S and/or 19S γ-globulin. It is known that the serum concentration of the 7S component much exceeds that of the 19S.

The presence of plasma cells containing rheumatoid factor was noted in small number in paravascular foci in the tissue of origin (the outermost zone) of rheumatoid subcutaneous nodules. Experience has been limited, however, to the study of nodules of long standing and has indicated only that an analysis of earlier lesions will be required for a better understanding of the pathogenesis of this type of rheumatoid granuloma which, in identical example, occurs only with rarity in the synovial and the capsular tissues in rheumatoid arthritis.

The characteristic apple-green staining in tissue specimens is well correlated with the occurrence of the rheumatoid factor in serum. The cellular factor was demonstrated in tissue specimens from ten patients who had active rheumatoid arthritis and latex fixation titers exceeding 1:2,560. Conversely, in all control subjects there was absence of cellular as well as circulating rheu-

matoid factor. These findings attest to the high selectivity of the staining reagent for rheumatoid factor. The relationship between the presence of cellular and circulating rheumatoid factor has, however, not yet been studied with individuals who show only weak latex fixation tests. However, in view of the high sensitivity as well as the adequate sampling inherent in serological tests, it might be anticipated that instances may occur in which no cellular rheumatoid factor is detected in synovial or lymph node biopsies despite the presence of a positive latex fixation test.

Lacking evidence to the contrary, it is reasonable to believe that the rheumatoid factor is not only contained in, but is actually produced by, plasma cells and germinal-center cells. The rheumatoid factor was detected in these cells in various stages of development and maturation, including the earliest. Secretion of the factor was suggested by the cellular polarity and the extracellular depositions which were observed. It is unlikely that a circulating macroglobulin such as rheumatoid factor could penetrate intact cells from the surrounding extracellular fluid. It is notable that prior treatment of serial sections with autologous or homologous (human) serum containing rheumatoid factor in high titer failed to modify the number and the types of cells specifically stained. The rheumatoid factor therefore is not adsorbed by plasma cells or germinal cells *in vitro* and such a deposition is unlikely *in vivo*.

The observations that cells of similar morphology are known to contain and produce antibodies (12), γ-globulin (9) of either 7S or 19S types, and, as shown by this study, rheumatoid factor, suggest the possible antibody nature of the factor. However, plasma cells are also known to produce protein of a non-antibody character, such as the myeloma proteins, and therefore this study neither confirms nor denies an antibody hypothesis. Nevertheless, a close relationship of the rheumatoid factor to an underlying mechanism in rheumatoid arthritis seems evident from the characteristic presence in the actively inflamed synovial membrane of plasma cells containing, and apparently forming and liberating, rheumatoid factor.

SUMMARY

Fluorescein-labelled aggregated human γ-globulin was found to react in precipitin-type tests with serums of individuals with rheumatoid arthritis. This reagent was also highly reactive and specific for the localization of rheumatoid factor in frozen sections of synovial membranes, lymph nodes, and subcutaneous nodules.

In synovial membranes from patients with active rheumatoid arthritis, rheumatoid factor was present in the cytoplasm of plasma cells at various stages of development and maturity. The appearance of the cytoplasm and the occasional presence nearby of extracellular particles suggested the possibility of a secretory process. All other cells were devoid of rheumatoid factor. Some

plasma cells contained 7S and/or 19S γ-globulin and many lacked detectable γ-globulin.

In lymph nodes from a patient with active rheumatoid arthritis, rheumatoid factor was present in approximately one in ten germinal centers as well as in internodular plasma cells. The rheumatoid factor was localized in the cytoplasm and the characteristic protoplasmic processes of the germinal-center cells. All other cells were devoid of rheumatoid factor. 7S and/or 19S γ-globulin was demonstrated in approximately eight in ten germinal centers in these lymph nodes.

Plasma cells with rheumatoid factor were also seen on occasion in rheumatoid subcutaneous nodules.

Tissue sections of comparable structure prepared from normal and pathological control material did not contain rheumatoid factor.

Staining for rheumatoid factor was blocked by pretreatment of sections either with unlabelled aggregated human γ-globulin or with rabbit antiserum against 19S human γ-globulin.

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EXPLANATION OF PLATES

PLATE 92

The illustrations are fluorescence photomicrographs of 4μ thick, frozen sections of tissues in rheumatoid arthritis stained with fluorescein-labelled proteins. The descriptions pertain to the specific staining of rheumatoid factor by fluorescein-labelled aggregated human γ-globulin, Figs. 1 to 15, 17 to 22, and 24; of 19S human γ-globulin by fluorescent rabbit antibody, Fig. 16; and of 7S and/or 19S human γ-globulin by fluorescent goat antibody, Fig. 23.

FIG. 1. Synovial membrane. An immature plasma cell with centrally located nucleus devoid of staining, and cytoplasm of homogeneous appearance containing rheumatoid factor (white areas). × 1000.

FIG. 2. Synovial membrane. A mature Marshalko-type, plasma cell with eccentric unstained nucleus, cytoplasm of indistinctly granular and homogeneous appearance containing rheumatoid factor, and crisp nuclear and rounded cellular boundaries. × 1000.

FIG. 3. Synovial membrane. A mature plasma cell with an extremely eccentric, unstained nucleus and cytoplasm containing rheumatoid factor. × 1000.

FIG. 4. Synovial membrane. A mature plasma cell with cytoplasm containing rheumatoid factor and showing an indistinctly granular appearance, less staining in the juxtanuclear region, and a few, barely perceptible small granules overlying the nucleus. × 1000.

FIG. 5. Synovial membrane. A binucleate mature plasma cell with cytoplasm containing rheumatoid factor. × 1000.

FIG. 6. Synovial membrane. Two mature plasma cells sharing cytoplasm and containing rheumatoid factor with a dense homogeneous perinuclear distribution in one cell and elsewhere in the cytoplasm appearing finely vacuolated. × 1000.

FIG. 7. Synovial membrane. Mature plasma cell with eccentric expanse of cytoplasm containing rheumatoid factor and showing wavy irregularity of the cell margin along one edge. × 1000.

FIG. 8. Synovial membrane. Mature plasma cell with cytoplasm containing rheumatoid factor and apparently giving rise to small extracellular globules and irregular masses of rheumatoid factor located adjacent to the cell. × 1000.

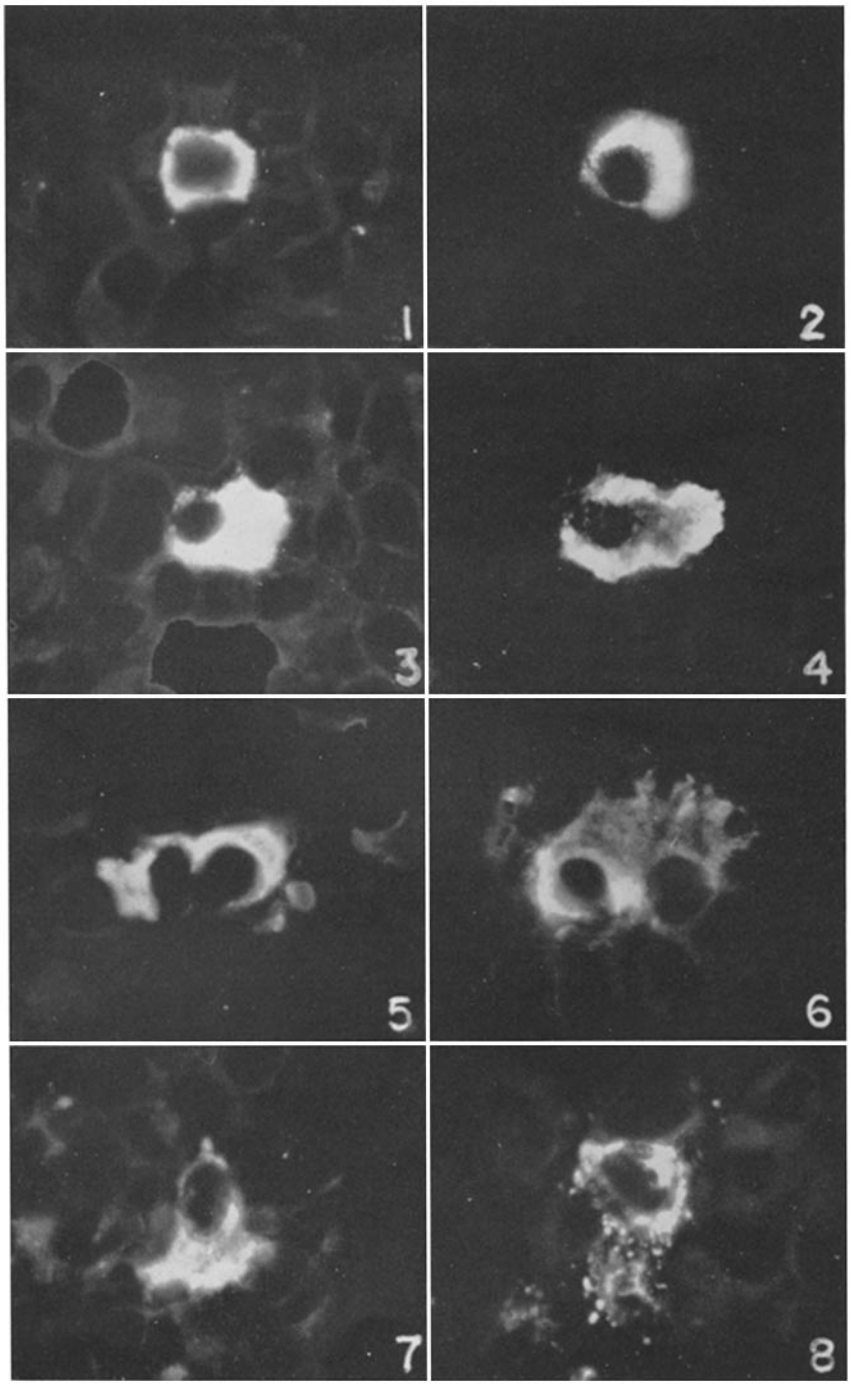

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PLATE 93

FIG. 9. Synovial membrane. Two mature plasma cells containing rheumatoid factor in Russell bodies which are small globules (in the earliest stage of resolution) occupying the cytoplasm although appearing less numerous in the juxtanuclear region. Each nucleus is eccentric and devoid of staining, and one nucleus has a polyhedral shape. × 1000.

FIG. 10. Synovial membrane. Russell body plasma cell with round and oval Russell bodies containing rheumatoid factor and appearing, at certain focal planes as bright equatorial rings with central, less stained regions, an effect attributable to failure of penetration of the fluor into the protein-rich spheres. The nucleus is eccentric and unstained. × 1500.

FIG. 11. Synovial membrane. Russell body plasma cell with crescent-shaped nuclear shadow and round and oval Russell bodies of ring-like appearance containing rheumatoid factor. × 1500.

FIG. 12. Synovial membrane. Russell body plasma cell with Russell bodies containing rheumatoid factor and obscuring the nucleus, which is located in the central grey area. × 1500.

FIG. 13. Synovial membrane. Russell body plasma cell with coalescent or confluent Russell bodies, no longer seen as discrete rings but containing rheumatoid factor. The nucleus is located near the concave border. × 1000.

FIG. 14. Synovial membrane. Three Russell bodies occurring free in the interstitial tissue space and containing rheumatoid factor. × 1500.

FIG. 15. Synovial membrane. Extracellular mass and plasma-cell residue containing rheumatoid factor. × 1000.

FIG. 16. Synovial membrane. Mature plasma cell with abundant, axially oriented cytoplasm containing 19S human γ-globulin and eccentric oval nucleus devoid of staining. × 1500.

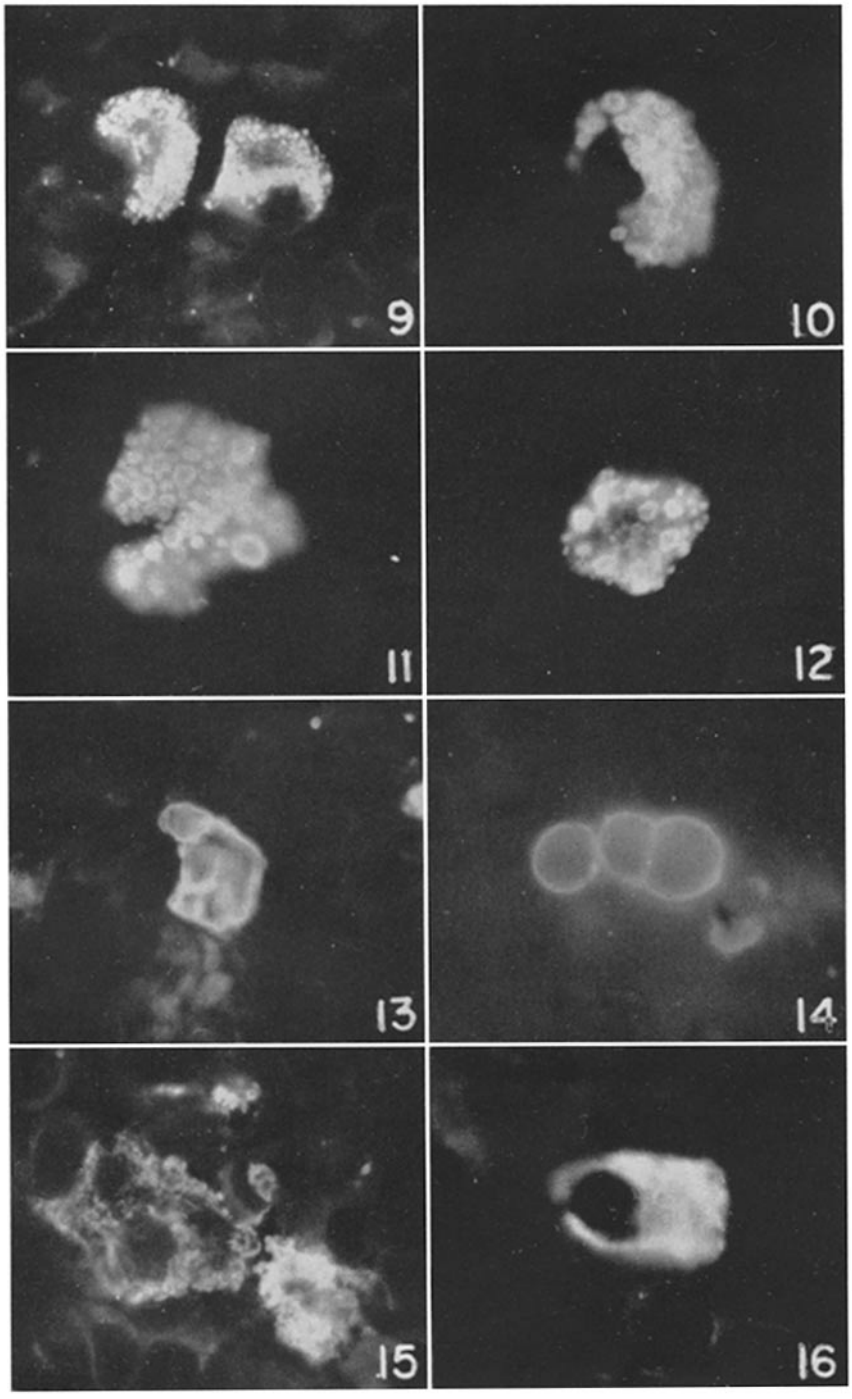

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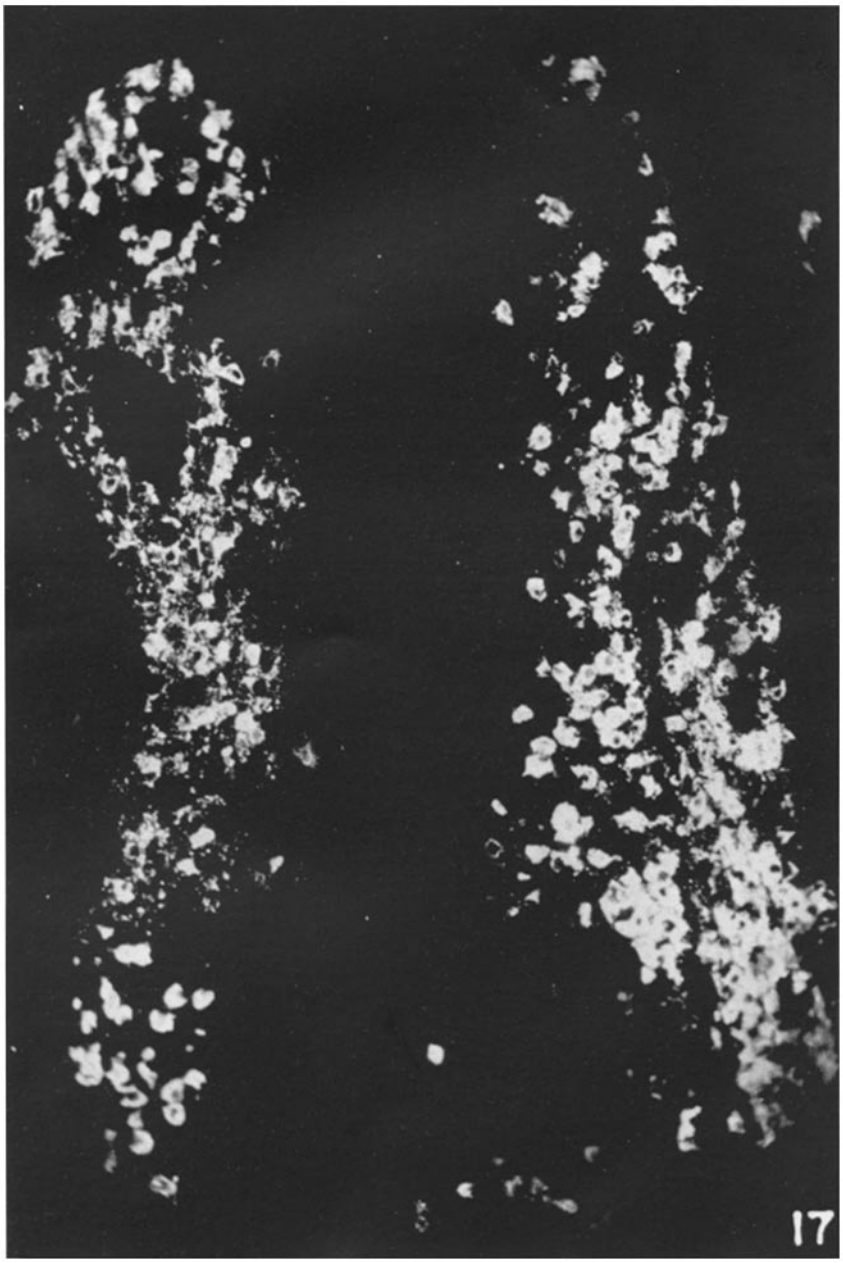
PLATE 94

FIG. 17. Synovial membrane. Two hypertrophic villi, vertically oriented, with their submesothelial inflammatory cores rich in plasma cells containing rheumatoid factor (white areas). × 245.

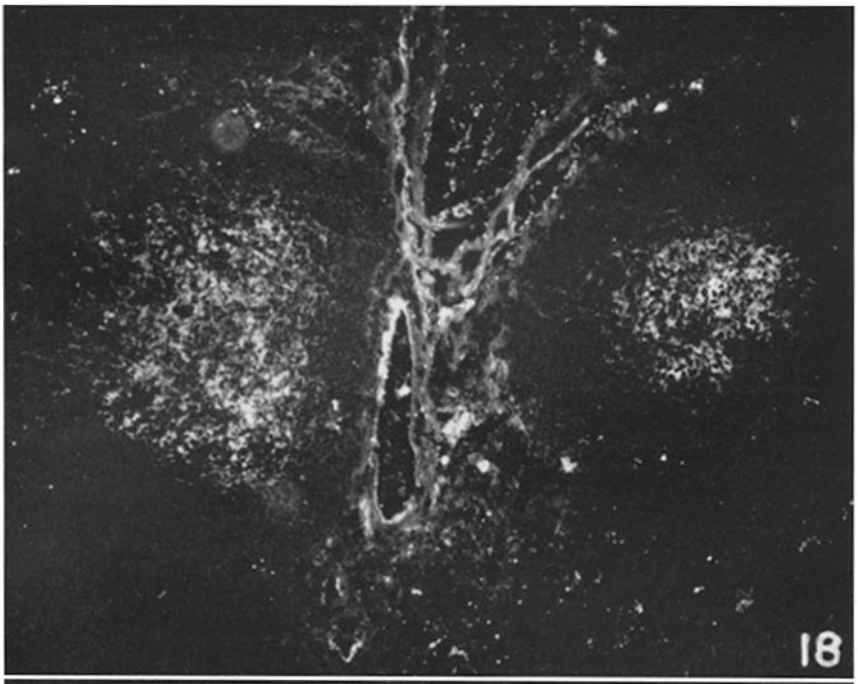


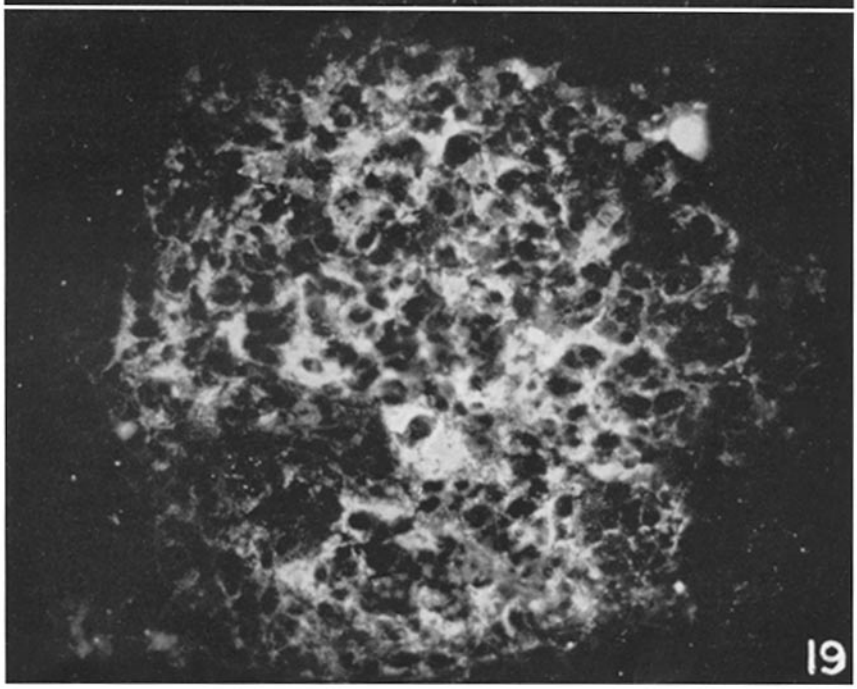
(Mellors *et al.*: Cellular origin of rheumatoid factor)

PLATE 95

FIG. 18. Lymph node. Two lymphoid nodules separated by autofluorescent, flat oval blood vessel and fibrous trabeculae, with the germinal centers alone containing rheumatoid factor and the surrounding mantles of mature lymphocytes devoid of staining. × 100.

FIG. 19. Lymph node. Germinal-center cells containing rheumatoid factor in their cytoplasm and cytoplasmic continua, with their nuclei, some other central areas, and surrounding mantle of mature lymphocytes devoid of staining. × 400.





(Mellors *et al.*: Cellular origin of rheumatoid factor)

PLATE 96

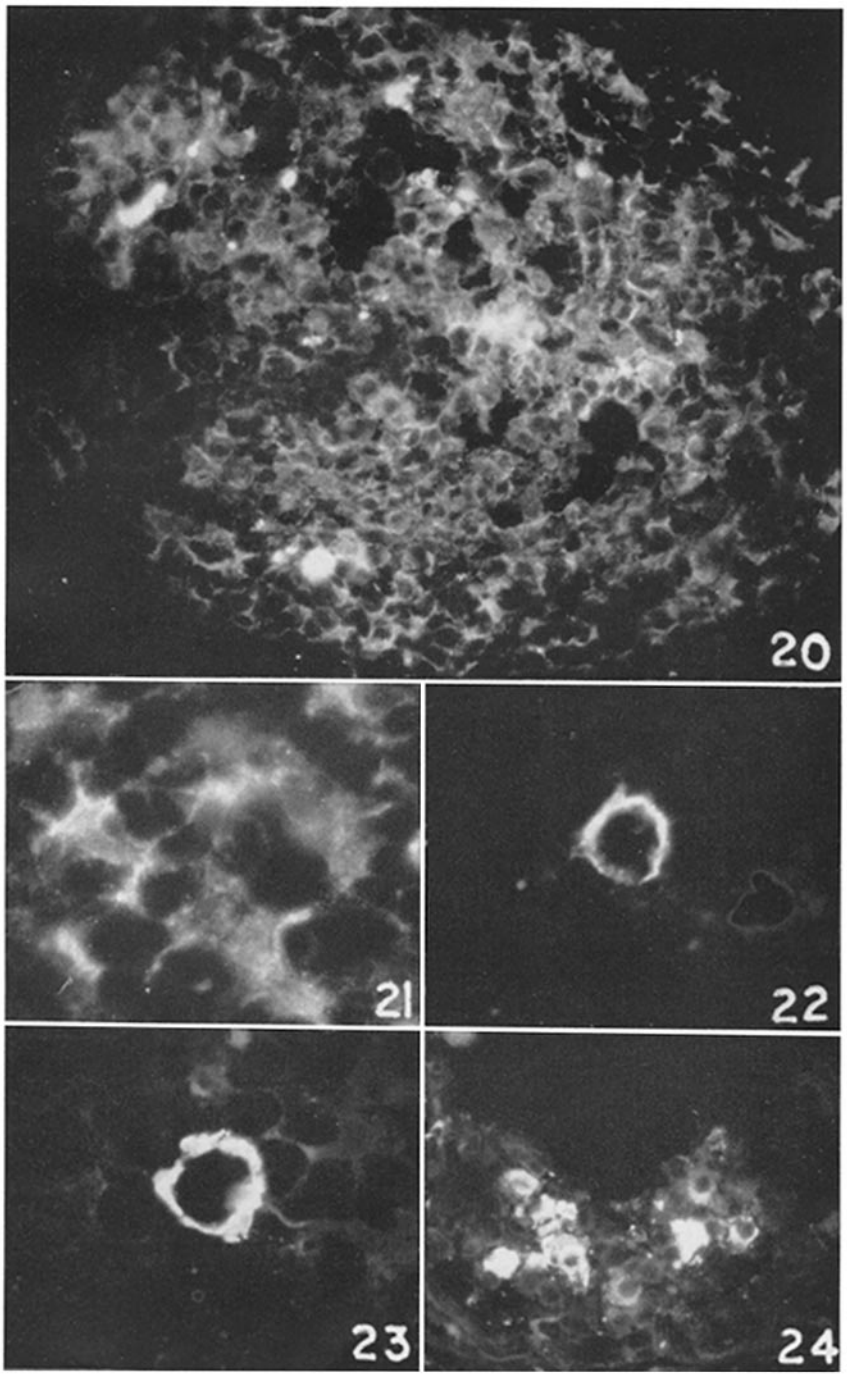
FIG. 20. Lymph node. Germinal center containing rheumatoid factor in lace-like distribution in cytoplasmic continua of germinal-center cells, with areas devoid of staining. Bright spherical and elliptical objects at margin are autofluorescent artifacts. × 400.

FIG. 21. Lymph node. Portion of germinal center showing indistinctly particulate and homogeneous distribution of rheumatoid factor in cytoplasm of germinal-center cells and in their cytoplasmic continua, with nuclei devoid of staining. × 1000.

FIG. 22. Lymph node. Immature plasma cell located in internodular medullary cord and containing rheumatoid factor in the cytoplasm, with large central nucleus devoid of staining. × 1000.

FIG. 23. Lymph node. Immature plasma cell located in internodular medullary cord and containing 7S and/or 19S human γ-globulin in the cytoplasm, with large central nucleus devoid of staining. × 1000.

FIG. 24. Subcutaneous nodule. Immature and mature plasma cells located in paravascular cluster in outer fibrous and inflammatory zone and containing rheumatoid factor in their cytoplasm. × 400.



(Mellors *et al.*: Cellular origin of rheumatoid factor)