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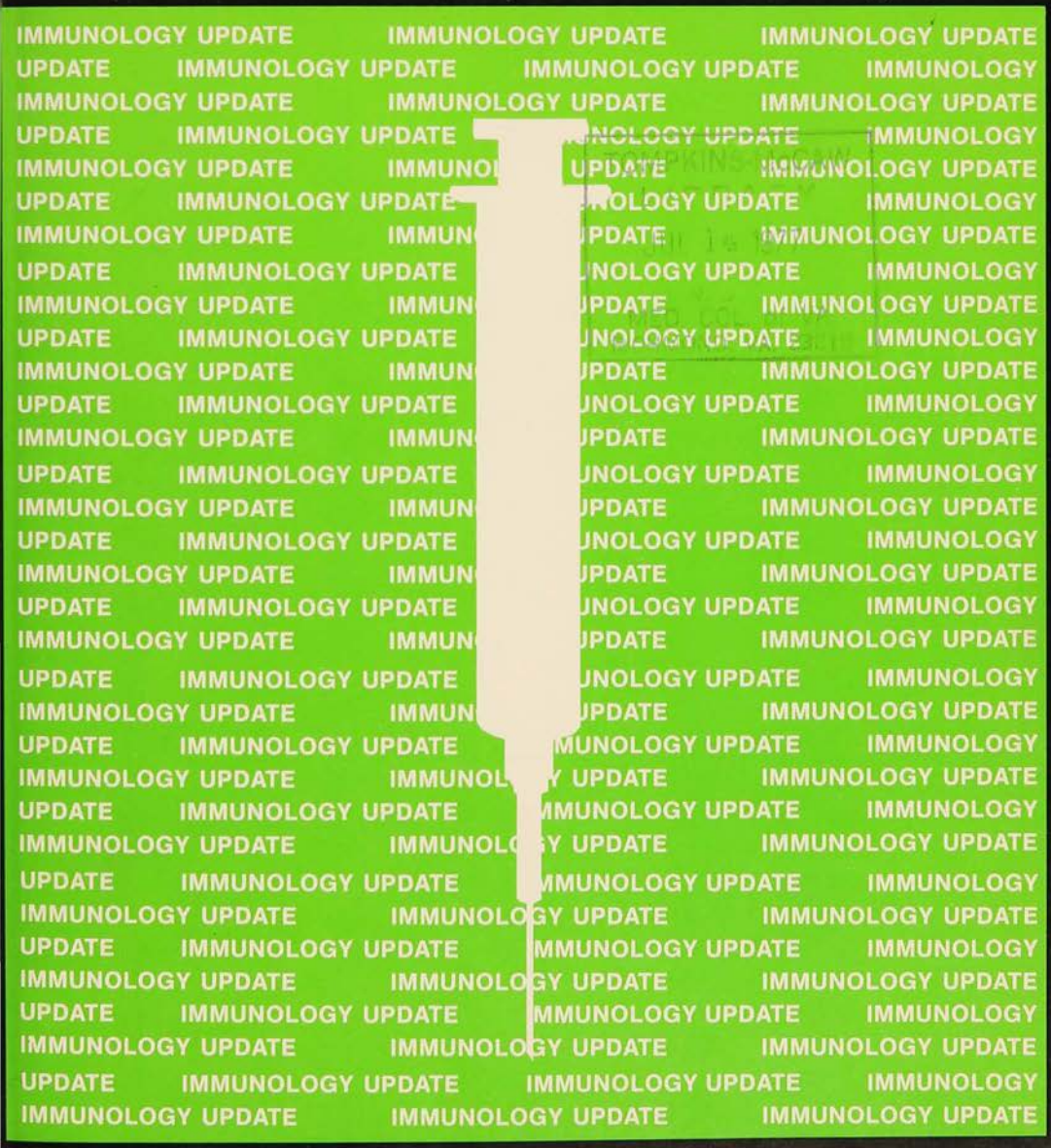

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CONTENTS

THE 48TH ANNUAL MCGUIRE LECTURE SERIES
Immunology Update

Presented by the Department of Continuing Education

DAVID B. WALTHALL, JR., M.D., *Guest Editor*

Printed by the William Byrd Press, Richmond, Virginia

INTRODUCTION

This issue of the *MCV Quarterly* is devoted to the 48th Annual McGuire Lecture Series held on February 12-13, 1977. The lectures, presented this year by the Department of Continuing Education, were designed to focus upon the ever-increasing amount of new information being generated in the field of immunology.

The program title, "Immunology Update," is indicative of our attempt to provide a comprehensive view of the latest research findings and developments and their implications for the clinical setting. Topics addressed included: (a) an overview of basic immunology; (b) a discussion of the clinical manifestations and management of immunological disorders; (c) presentations on how to do office work-ups of immunological problems; and (d) discussions on the use of immunological diagnostic techniques.

We were fortunate to have as our McGuire Lecturer, Richard Hong, M.D., Professor of Pediatrics, University of Wisconsin School of Medicine, Madison, Wisconsin. Dr. Hong is recognized internationally as one of the outstanding authorities on immunology. Nine other highly capable physicians comprised an outstanding faculty. We are in their debt for providing a series of lectures that proved attractive to physicians, as was reflected by the large attendance, and one that was stimulating and informative.

DAVID B. WALTHALL, JR., M.D.
Assistant Dean for Continuing Medical Education

The Emerging Clinical Usefulness of Complement Measurements*

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Not many years ago the main purpose of "complement" seemed to be to drill holes in sheep erythrocytes. In the classic experiment which was part of every medical student's microbiology laboratory experience, a magic stuff called complement, somewhat mysteriously obtained from guinea pigs, was either "fixed" or not "fixed" and the sheep cells either not lysed or lysed accordingly.[1] That was about all there was to know about complement, and all one needed to know.

Today the term "complement" embodies a group of plasma proteins which react in a complex sequence to mediate a variety of inflammatory effects, including changes in vascular permeability, the attraction of polymorphonuclear or mononuclear leukocytes, the enhancement of phagocytosis, and damage to cell membranes and osmotic lysis such as the sheep erythrocyte suffered in the complement fixation test. These complement proteins rival the intrinsic coagulation scheme in complexity and resemble it in mechanism, that is, the complement proteins normally circulate in the plasma in an inactive or precursor form, and when appropriately stimulated, usually during an immunologic reaction, become transformed into active enzymes, or proteases. These proteases act upon their natural substrates (other members of the complement system) in an orderly and predetermined sequence of limited proteolytic reactions which are often compared to a "cascade" or "waterfall." Cleavage of one component leads to the activation of the next component, and so forth. Natural inhibitors or inactivators also present in plasma serve to modulate or damp this cascade system and prevent its getting out of control. In fact, congenital deficiency of one of these inhibitors, the C1 Inhibitor, leads to uncontrolled activation of the system and recurrent swelling of the subepithelial tissues of the skin, respiratory and gastrointestinal tracts.[2]

Biochemical Pathways.[3-5]

Two pathways for activation, the classic and alternative (properdin), initiate the terminal attack sequence which elaborates most of the biologic activities associated with complement. The classic pathway is activated by immune complexes containing IgG or IgM immunoglobulins and their associated antigens. The properdin pathway is activated by certain kinds of repeating polysaccharides such as pneumococcal polysaccharide, or the bacterial lipopolysaccharide of gram-negative endotoxin; immune complexes containing IgA may also activate the properdin system.

Regardless of which pathway is activated, both result in the cleavage of C3 and C5. Peptides released from these components, C3a and C5a, are anaphylatoxins capable of releasing histamine from mast cells and thereby influencing local vascular permeability. C5a also releases lysosomal enzymes and presumably other granular contents from polymorphonuclear leukocytes. Both C3a and C5a have chemotactic activity as well, the latter being more active in most systems; the trimolecular complex formed from C5, C6, and C7 is also chemotactic. Immune complexes to which complement, especially

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C3b, has become bound adhere to polymorphonuclear neutrophils, mononuclear cells and B-lymphocytes. Although the functional significance of these binding phenomena is not yet entirely clear, enhanced phagocytosis by mononuclear or polymorphonuclear cells is certainly one consequence. Formation of a multimolecular complex involving C5, C6, C7, C8, and C9 leads to the membrane damage and osmotic lysis which have become the hallmarks of complement activation.

Metabolism of Complement Proteins.

One result of the proteolysis of the complement proteins during their activation is that they subsequently become recognizable as "altered" or "foreign" by the body and are rapidly cleared from the circulation. Although some compensatory increases in synthesis may occur, the result is usually a fall in plasma level. Thus, an ongoing immunologic event (or disease) which is activating the complement system in vivo may be manifested as a fall in the serum or plasma level of one or more of the complement proteins. In reverse fashion, as the complement-activating stimulus or disease abates, this may be paralleled by a return towards normal in the complement levels.

Determinations of Complement in the Clinical Pathology Laboratory.

In principle, there are two ways of measuring complement: (1) by its activity in the reaction it catalyzes, for example, the total hemolytic complement or CH50, which measures the result of the interaction of all nine of the classic complement components or (2) by its antigenicity, as a protein in an immunoassay which takes advantage of the complement protein's capacity to react with monospecific antibody directed against it. In practice, although total hemolytic complement or CH50 determinations are available in some institutions, immunoassays (usually radial immunodiffusion) are most frequently available. Materials for these are offered in the form of kits for use in the clinical pathology laboratory by a number of commercial suppliers.

A few comments are in order about the radial immunodiffusion determinations for complement components available in most hospitals.

1) Radial immunodiffusion, by its very nature, is not nearly as precise a determination as most physicians have come to expect from clinical laboratories. Under the best conditions, the coefficient of variation of the test is likely to be 8% or greater, more than twice that of commonly available clinical chemistry or hematologic procedures. Thus in interpreting the results of the test, the physician must take into account this reduced precision, for example, a "fall" in C3 level from 145 to 130 mg/100 ml from one day to the next may reflect only laboratory variation.
2) There are no widely available standards, so that considerable variation in absolute values obtained by kits from different suppliers or even in lots of kits from the same supplier may be observed. This should not be a problem if results are referred to a normal range collected at the institution in which the test is being performed, and if appropriate internal standards, maintained at the institution, are assayed in parallel with the test samples. If the "normal ranges" provided with the kits are accepted as verbatim, and if independent checks of the performance of the kits are neglected, then unreliable data may result.
3) As for other plasma proteins, the range of normal for complement proteins is quite broad, usually in the vicinity of ± 50% of the mean value for the population. Thus changes in levels in a single patient over a period of time may often be more helpful and easier to interpret than are comparisons with some absolute range of normal. For example, a patient with suspect systemic lupus erythematosus whose C4 level fell from 70 mg/100 ml to 30 mg/100 ml within two weeks might be cause for alarm, even though the latter value was still "within the range of normal."
4) The most widely available test for a complement component (C3) is not necessarily the most desirable. Its availability is directly related to the fact that C3 is by far the most plentiful of the complement components, the easiest to purify, and therefore the easiest to make antibody against for use in a radioimmunoassay. Measurements of C3 were therefore widely available from commercial sources several years in advance of measurements of other components. Most workers would agree today, however, that measurements of C4 are likely to be more sensitive to minor episodes of in vivo complement activation, and that a good "routine" complement screen would include measurements of C4, C3, and possibly CH50.

5) The immunoassays do not distinguish between native protein and that which has participated in complement activation and lost its activity but not its immunogenicity. They are reliable, therefore, only in instances in which substantial amounts of cleaved, inactive protein would not be expected. Altered complement proteins are cleared within a few hours from the plasma space, and the finding of altered inactive protein in plasma requires special techniques to detect the small amounts which are present. In contrast, however, altered inactive protein may persist for much longer times in joint spaces or pleural spaces, so that radial immunodiffusion determinations of C3 or C4 in synovial or pleural fluid are of very little value. Recent studies have shown that measurements of C4 in cerebrospinal fluid are similarly of little diagnostic value with respect to the presence or absence of central nervous system involvement in systemic lupus erythematosus.

Clinical Significance.

Given the knowledge that the complement system may be activated in vivo by immunologic diseases, and that simple and reliable methods for measuring complement levels are now widely available, how can the practitioner best use this information? What are the diseases in which complement measurements are likely to be of help, either in diagnosis or in following the course of the patient? The answer is simple: any disease in which the physician suspects that circulating immune complexes may be playing a pathogenetic role. A few of them are listed in the Table.

Systemic lupus erythematosus is the disease which is perhaps most commonly associated with hypocomplementemia, but it is well to bear in mind that other diseases in which circulating antigen-antibody complexes may be found may also give rise to hypocomplementemia. Among these would be included any cause of "chronic antigenemia," for example, subacute bacterial endocarditis, hepatitis B surface antigenemia, infected atrioventricular shunts, recurrent gram-negative sepsis, recurrent viremias such as dengue hemorrhagic fever, or recurrent parasitemia, such as falciparum malaria. Other diseases of unknown etiology may be associated with hypocomplementemia, such as essential mixed cryoglobulinemia, or certain kinds of nephritis (which in contrast to all the diseases mentioned above which are likely to have low C4, and sometimes low C3, usually have only low C3, suggesting direct alternative pathway activation). The symptom of "angioedema" is infrequently associated with hypocomplementemia, but a screening test should be done for C4, which is low in almost all cases of *hereditary* angioedema, especially since very effective treatment for this disease is now available.

In most instances in which hypocomplementemia is found in association with the disease, improvements in complement levels are often early and reliable indices that the disease is ameliorating either spontaneously or as a result of therapy.

Thus, by applying his understanding of "complement fixation," obtained in the classical microbiology laboratory experiment, to human diseases in which "complement fixation" appears to be occurring in vivo, today's physician has achieved a useful diagnostic tool and therapeutic index in the measurement of complement components in disease.

TABLE

Diseases in which Complement Determinations May Provide Useful Diagnostic or Therapeutic Information

Systemic lupus erythematosus
Rheumatoid arthritis (with systemic vasculitis)
Hypersensitivity angiitis
Angioedema
Glomerulonephritis
Subacute bacterial endocarditis
Hepatitis
Essential mixed cryoglobulinemia

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Immunology and Diseases of Connective Tissue*

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Since immune responses play a major role in the development of connective tissue diseases, it is not surprising that a number of laboratory studies reflect these responses. Prior to the 1940s when rheumatoid and LE factors became widely known, one relied mainly on erythrocyte sedimentation rate and serum electrophoresis to identify protein abnormality. Elevated sedimentation rate depends on rouleaux formation, and rouleaux formation is dependent upon large asymmetric molecules of fibrinogen and gamma globulin in plasma. The demonstration of gamma globulin has become the cornerstone of the immunologist's edifice. It is amazing to see how the subspecialty of immunology has mushroomed to involve the many facets of disease processes such as connective tissue diseases, skin diseases, gastrointestinal diseases, renal diseases, and cancer. More recently, immune deficiency diseases have included the pediatrician in the ever-enlarging field of immunology as has the modern-day discovery of human leukocyte antigen (HL-A) testing and tissue typing included the geneticist.

In this paper, I will discuss four connective tissue diseases—rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, and mixed connective tissue disease syndrome—and the role immunological processes play in their pathogenesis. I will also comment upon certain laboratory tests that use immunological methods in making a diagnosis.

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I. Rheumatoid Arthritis.

It is believed that an immune process plays a role in the perpetuation of rheumatoid arthritis and there are at least five items which may provide evidence for this hypothesis.

1. Lymphoid cell infiltration of the synovial membrane with follicle formation. Note similarity to architecture of lymph node (Figs 1*A* and 1*B*).
2. Local synthesis of IgG and rheumatoid factor by plasma cells in the synovium as demonstrated by Smiley et al.[1]
3. Decreased synovial fluid complement in some cases, and in other cases decreased serum complement as demonstrated by Ruddy and Schur.[2,3]
4. Presence of IgG and IgM and complement components in the synovial lining cells and in remote sites of tissue damage indicating that some immunological process is taking place also at distant sites.[4]
5. Presence of rheumatoid factor components and complement in leukocytes of synovial fluid cells.[5]

The current concept is that IgG is produced in rheumatoid synovial membrane by an unknown stimulus; this in turn stimulates production of IgM by plasma cells of the synovium and lymph nodes. Why IgG, which is a product of the human system, should serve as an antigen for IgM antibody production, is unknown; however, immune events may depend upon circulating complexes which may or may not be important in pathogenesis. There are some who feel that rheumatoid factor plays a protective role similar to the antistreptolysin titer in streptococcal infection,

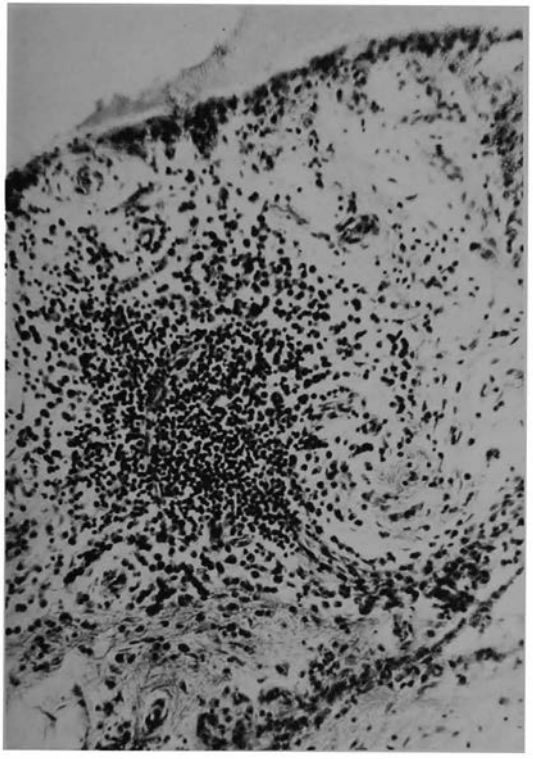

Fig 1*A*—Microscopic view *(medium power)* of synovial membrane showing "nesting" of lymphocytes in perivascular area of synovium.

while others feel that in certain systems it plays a part in the inflammatory mechanism. The basic pathology in these diseases seems to be vasculitis; hence the term collagen-vascular disease arose, which is a misnomer, since collagen is not the only connective tissue involved. In rheumatoid arthritis with high rheumatoid factor titers and many subcutaneous nodules, one is more likely to see those with severe disease: peripheral neuritis, vasculitis, and leg and fingertip ulcerations. In these patients one may find evidence of antigen-antibody complement complex deposition in the vessels leading to the ulcers and in the perineural vessels of the peripheral nerves (Figs 2*A*, 2*B* and Figs 3*A*, 3*B*).

There are a number of immunological tests available to make a diagnosis of rheumatoid arthritis. The three currently in use at the Medical College of Virginia are the slide latex test, sensitized human cell test (SHC), and the sensitized sheep cell test (SSC). The latex test is the most sensitive and the sheep cell test is the most specific for the presence of rheumatoid factor. Sometimes there are false-positive latex tests in older patients and in patients with large amounts of gamma globulin present. The SSC may be positive in low titers and in other connective tissue diseases. In general, all three tests employ the same principle. The particle, whether it be latex, human red cells, or sheep cells, is coated with IgG from one source or another, which will agglutinate in the presence of rheumatoid factor. If the sheep cell test is positive in a significant titer, the chances are over 90% that the patient has rheumatoid arthritis. It should be remembered that a large number of patients do not show rheumatoid factor, and these patients are classified as seronegative rheumatoid arthritis patients (Fig 4 and Fig 5).

We can identify rheumatoid factor in serum, demonstrate that it is produced by plasma cells in the synovium, and can state that in certain cases of rheumatoid arthritis it plays a pathogenetic role.

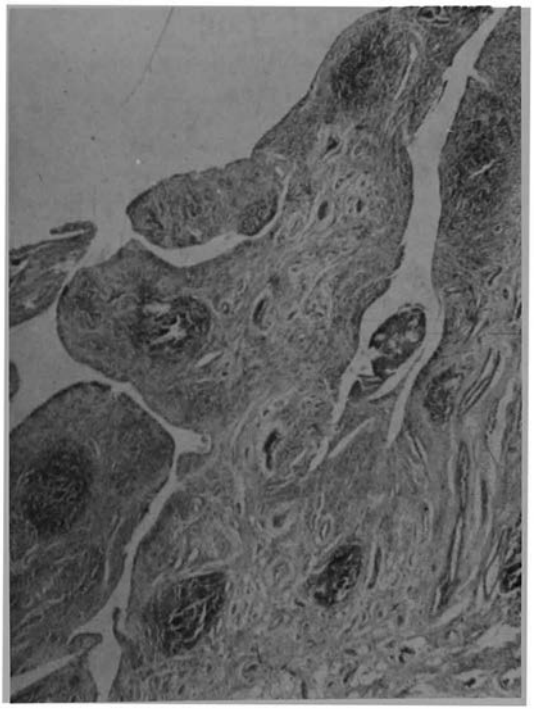

Fig 1*B*—View *(lower power)* showing follicle formation within the synovium. Note similarity to microscopic picture of lymph node. This is thought to be the site of rheumatoid factor synthesis.

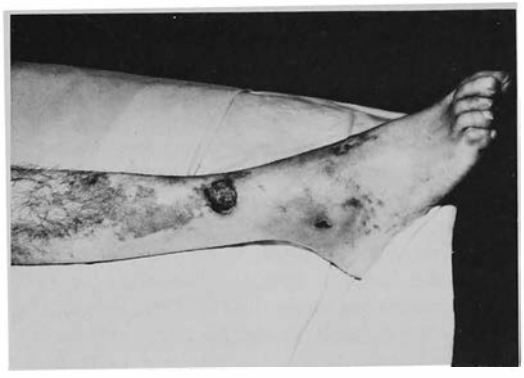

Fig 2*A*—Indolent leg ulcer in a 64-year-old male with rheumatoid arthritis which ultimately required below-the-knee amputation.

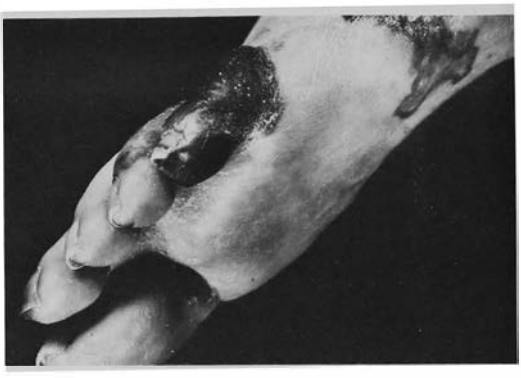

Fig 3*A*—Digital vasculitis in a patient with rheumatoid arthritis with gangrenous changes.

II. Ankylosing Spondylitis.

We generally categorize this as a separate disease entity from rheumatoid arthritis, although it has many similarities to that disease. There are, however, five specific differences:

1. Sex and age of onset—more common in young males.
2. Spine and girdle joints involved as opposed to peripheral joints, although peripheral joints may be involved in ankylosing spondylitis.
3. Pathology is that of ankylosis, rather than bone destruction as seen in peripheral arthritis.
4. Absence of rheumatoid factor.
5. Presence of a high incidence of HL-A B-27 antigen.

HL-A stands for Human Leukocyte Antigen, and B-27 refers to its genetic locus on the chromosome. All of this is an outgrowth of tissue typing necessary in renal transplantation. We have known for some time that ankylosing spondylitis patients have a tendency to familial clustering, and there has been a low incidence of spondylitis in American and African blacks. In 1949, Toone reported studies from the McGuire Veteran's Hospital indicating the paucity of blacks who suffered from ankylosing spondylitis.[6] Baum later confirmed this in a larger group of patients from a Veteran's cooperative study (Table 1).[7] Schlosstein et al, and Brewerton et al, had found that there was a high correlation between the presence of HL-A B-27 antigen in ankylosing spondylitis—as high as 88% to 96%—and normal (8% in a controlled Caucasian population) (Table 2).[8,9] There

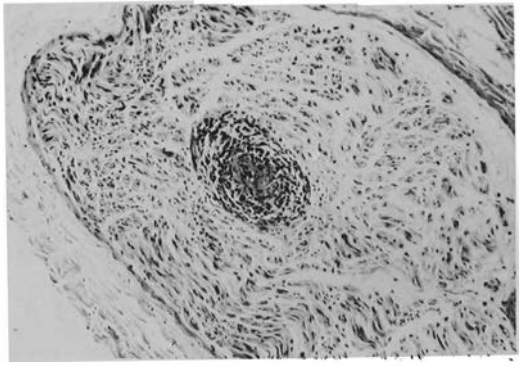

Fig 2*B*—Area of vasculitis (*medium power*) in the center of a nerve trunk of the lower extremity. Note perivascular changes in the nutrient artery of the trunk.

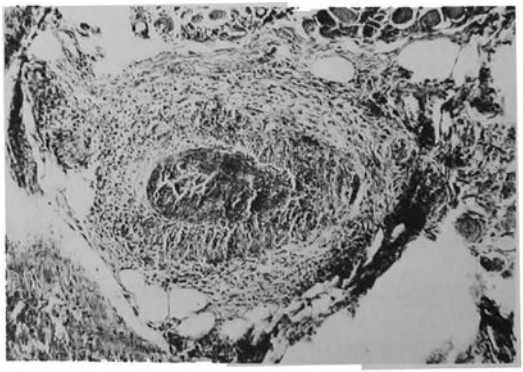

Fig 3*B*—Vasculitis and perivasculitis (*medium power*) of the necrotizing variety showing obliteration of the artery of the foot.

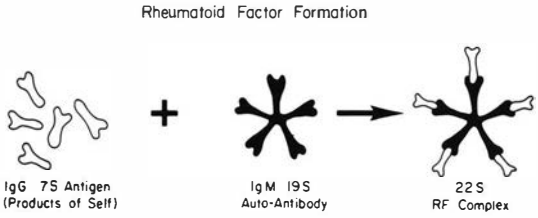

Fig 4—Rheumatoid factor formation. Rheumatoid factors are antibodies which react with other immunoglobulins to form a rheumatoid factor complex in serum. 7S refers to sedimentation constant in the ultracentrifugation analysis (Svedberg units).

are other diseases which have B-27 as a genetic marker. These include Reiter's syndrome, acute anterior uveitis, psoriasis with spondylitis, and others.

To better understand the immunological processes in developing a test such as the lymphocyte microcytotoxicity test (LMCT), it is important to understand the mechanics of the HL-A testing procedure. The LMCT is performed approximately as demonstrated in Figure 6.

1. Specific HL-A B-27 antiserum from multiparous women, or from those who have undergone numerous transfusions as a source of developing B-27 antibodies, is used.
2. The patient's lymphocytes to be tested for B-27 antigen are prepared and mixed with the antisera and rabbit complement in the chamber wells.

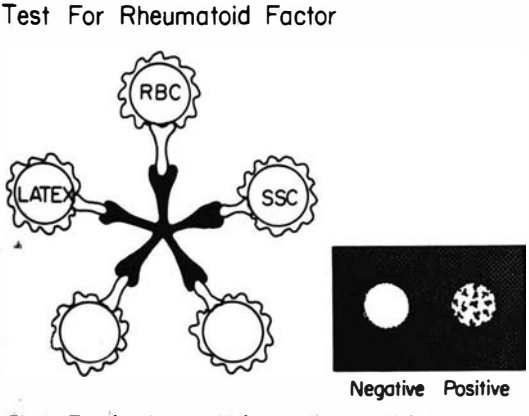

Fig 5—Test for rheumatoid factor. Rheumatoid factor complex will agglutinate either latex particles, human RBC or sheep RBC when sensitized with IgG. In the square at lower right the well on the left shows a negative test and on the right a positive test with latex particles.

3. The addition of trypan blue dye will stain the interior of lymphocytes which have been lysed by the antigen-antibody complement reaction. These lymphocytes are then counted under inverted phase microscopy to determine if the number of lysed cells is sufficient to make a specific identification of B-27 antigen.

One may conclude then that the HL-A B-27 antigen is a genetic marker for the development of certain diseases in which spondylitis seems to be a common denominator. However, the part this marker plays in the pathogenesis of the disease is unknown.

III. Systemic Lupus Erythematosus (SLE).

There is no other connective tissue disease which has evoked more interest among immunologists than lupus; it might be labeled "the immunologist's delight." The ability to diagnose this disease stemmed from the Hargraves' LE cell test in 1948 through various patterns of antinuclear antibody (ANA) testing, through the LE band test, and through the "lumpy-bumpy" deposits in the glomeruli.

If one understands the basic concept of production of experimental serum sickness, which was so well demonstrated by Dr. Frank Dixon with the formation of immune complexes, one might understand more clearly the pathogenesis of such diseases as glomerulonephritis, rheumatic fever, SLE, and perhaps even rheumatoid arthritis (Fig 7).[10] The parallel between these findings in experimental serum sickness and human SLE and the occurrence of a wide range of autoantibodies against nuclear and tissue antigens, provides strong support for an immunological process in SLE. These antibodies include anti-DNA antibodies, anti-DNA histone antibodies, anti-ribonucleoprotein (RNP) antibodies, anti-Smith (Sm) antibodies, and a host of others. The demonstration of DNA and anti-DNA antibodies in the

TABLE 1
Racial Aspects of Ankylosing Spondylitis*

1. Random study McGuire VA Hospital: 26 white, 3 black.—Toone 1949
2. Combined VA Hospital study, 301 patients: 10% black.—Baum 1971
3. HL-A 27 absent in Black Africans.
4. HL-A 27 only 4% in Black Americans.
5. HL-A 27 present in 8 of 10 Black American spondylitics.

*The above figures demonstrate the relationship of HL-A 27 and ankylosing spondylitis on Black Americans.

TABLE 2
HL-A 27 in Rheumatic Disease Patients*

Condition	Presence of HL-A 27	Percentage	Source
Normal	119/1456	8%	Russell, 1972
			Brewerton, 1973
			Schlosstein, 1973
			White, 1972
Rheumatoid arthritis	10/119	8%	Schlosstein, 1973
Gout	6/66	9%	Schlosstein, 1973
Ankylosing spondylitis	35/40	88%	Schlosstein, 1973
	72/75	96%	Brewerton, 1973
Reiter's syndrome	25/33	76%	Brewerton, 1973
Acute anterior uveitis	26/50	52%	Brewerton, 1973
Psoriasis	9/156	6%	White, 1972
	6/44	14%	Russell, 1972
Psoriasis/spondylitis	10/14	71%	Metzger, 1974

* A compilation of rheumatic diseases and HL-A 27 shows its strong association with ankylosing spondylitis, Reiter's syndrome, and psoriatic spondylitis.

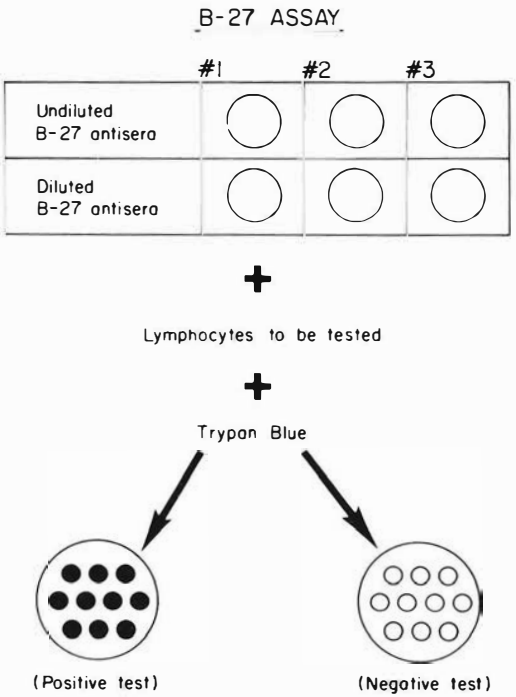

Fig 6—Lymphocyte microcytotoxicity test for B-27 antigen. Lymphocytes to be tested for B-27 antigen are added to each of three donor wells of diluted and undiluted specific B-27 antisera. With addition of complement and trypan blue dye, cells will lyse and stain positively with blue dye if B-27 antigen is present.

human kidney in SLE reinforces the concept that SLE is an example of an immune-complex deposition disease involving autoantibodies.[11,12]

Prior to the widespread use of antinuclear antibody testing for the diagnosis of SLE, one relied primarily on the demonstration of hematoxylin bodies in fixed tissue, or the presence of LE cells in the serum. Hematoxylin bodies are globular masses of nuclear material which stain blue with hematoxylin and eosin (H & E) stain and are histochemically identical with the inclusion body of an LE cell. Severe vasculitis is the hallmark of SLE, and fibrinoid deposits in vessels have been shown to be composed chiefly of DNA, anti-DNA, and complement components. The use of various immunological tests to determine the presence of ANA have taken the place of the LE cell test in making the diagnosis of SLE. The method used here is the indirect immunofluorescent antibody test, which employs mouse liver cells as the source of nuclear antigen. If antinuclear antibodies are present, various patterns of immunofluorescence will appear, depending on what type of antinuclear antibody is present in the patient's serum. The patient's serum to be tested is added to the mouse liver cells and will adhere to the nuclear antigen even when washed. When anti-human gamma globulin, which is commercially prepared and stained with fluorescin is added, a bright apple-green fluorescence will appear in the preparation when viewed under ultraviolet light (Fig 8). We have come to recognize four different fluorescent staining patterns, which may help in differentiating different patterns of disease expression in lupus. These include the homogeneous or diffuse pattern, indicating antibodies to

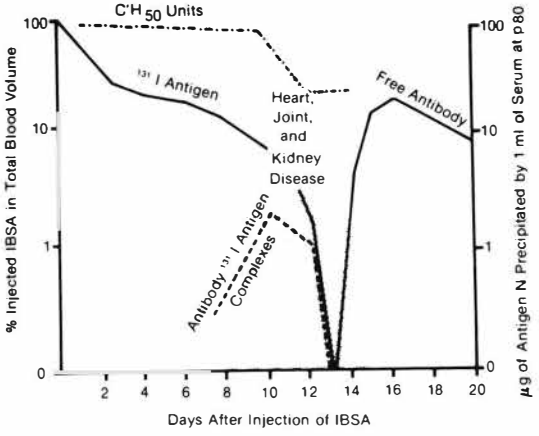

Fig 7—Circulating BSA-anti-BSA complexes: development of lesions. Correlation between formation of immune complexes with bovine serum albumin and appearance of lesions of heart, joints, and kidneys of rabbits with experimental serum sickness induced by injection of tagged BSA.

single- or double-stranded DNA; the peripheral or ring pattern, usually indicating the presence of antibodies to double-stranded DNA with active lupus present, usually with renal involvement; the nucleolar pattern, in which the nucleoli of the mouse liver cells take the stain, can be found in patients with systemic sclerosis or Sjögren's syndrome; and the speckled pattern, which stains for ribonucleoprotein and has a fibrillar appearance in the nucleus and is found in mixed connective tissue disease syndrome. It should be emphasized that these staining patterns and the statements regarding them are generalizations and should not be interpreted as explicit evidence in these cases. Sometimes we see a mixture of patterns, which may confuse the issue (Fig 9).

In some cases of SLE, where a drug such as hydralazine or procainamide is suspected in producing the LE phenomenon and antinuclear antibodies, a hemagglutination test or agar gel precipitation test using single- or double-stranded DNA may be used. Antibodies to single-stranded DNA are usually present in drug-induced SLE, whereas antibodies to double-stranded DNA may indicate active lupus with renal involvement. The ANA test is superior to the LE cell test in making a diagnosis in SLE because only 75% to 90% of patients with active SLE will have positive LE cell tests. Almost all patients with lupus will have a positive ANA test.

The conclusion here is that SLE is the immunologist's disease—many types of autoantibodies are present, which can be demonstrated by various immunodiffusion and immunofluorescent techniques. These autoantibodies are thought to play a specific role in the pathogenesis of SLE, particularly where there is renal involvement.

IV. Mixed Connective Tissue Disease (MCTD). The introduction of the term *speckled pattern* of ANA immunofluorescence and the presence of ribonucleoprotein and Smith antigens opens the door to mixed connective tissue disease syndrome.

The clinical picture of MCTD reveals it as a syndrome consisting of many of the features of the connective tissue disorders including rheumatoid arthritis with arthritis and arthralgias and rheumatoid factor, SLE with skin rashes and speckled pattern of antinuclear antibody immunofluorescence, progressive systemic sclerosis with Raynaud's phenomenon and thickening of the skin, and polymyositis with

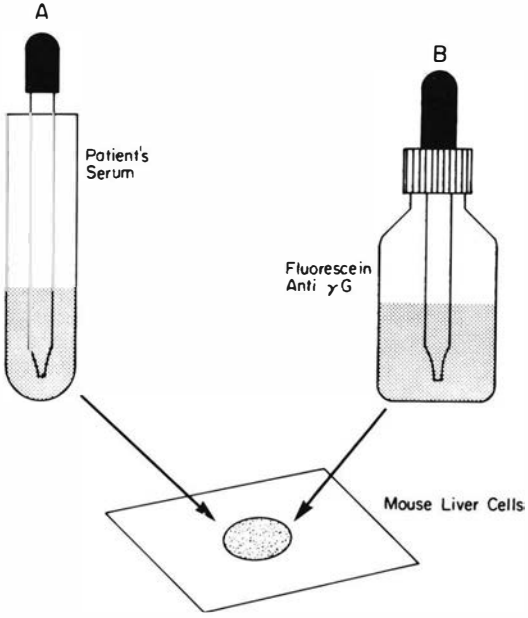

Fig 8—Indirect immunofluorescent staining for antinuclear antibodies. Mouse liver cells are used as the substrate for source of nuclear antigen. The patients' serum (*A*) containing antinuclear antibodies binds to the nuclei leaving a coating. After washing, the preparation is incubated with an anti-human gamma globulin (*B*) which binds only at the site where antinuclear antibody is bound. Nuclei exposed to SLE serum will fluoresce when examined under the fluorescence microscope.

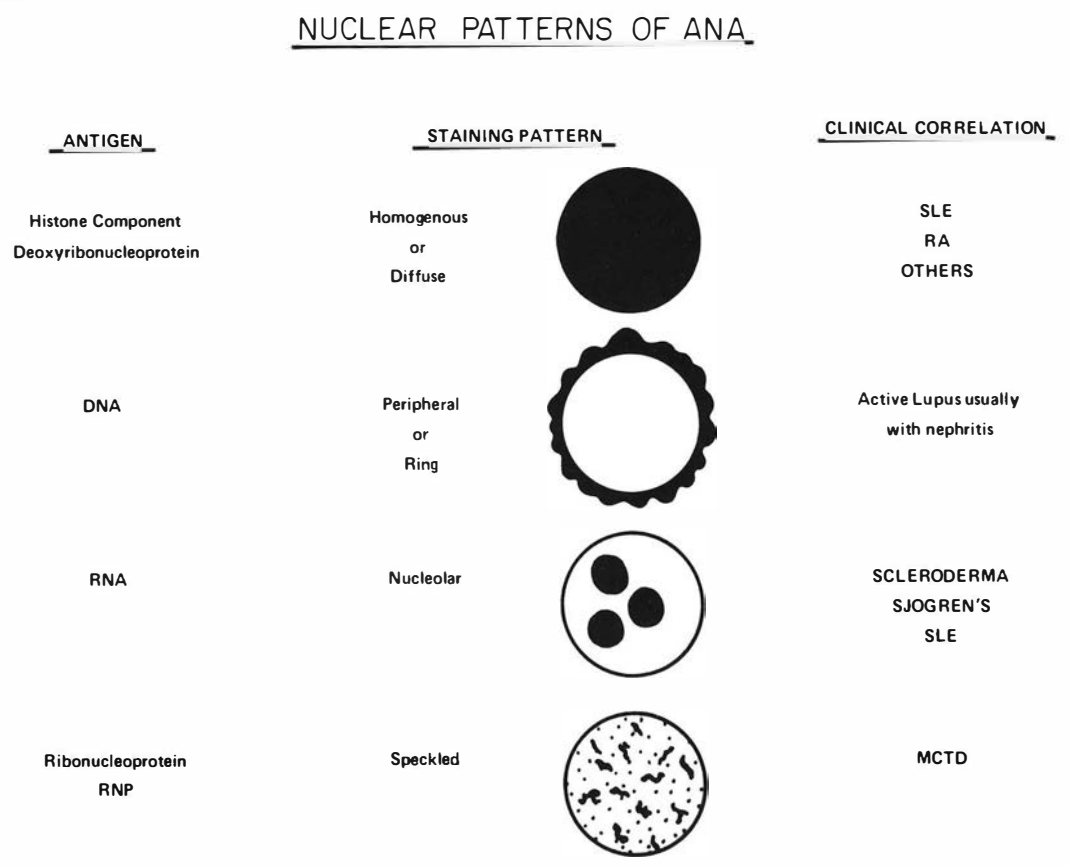

Fig 9— Different patterns of ANA testing. Antigen, pattern of fluorescent staining, and clinical correlation in the four types of nuclear antibody tests.

myopathy and muscle enzyme changes. In other words, it is a "mishmash" of the connective tissue diseases, but generally patients are thought to have a better prognosis with this syndrome and as a rule do not have severe renal or central nervous system (CNS) involvement as those with systemic lupus.

Concerning the diagnosis of MCTD, Gordon Sharp at the University of Missouri, who has done much work in this disease, feels that a diagnosis can be made if the clinical picture fits and one can demonstrate antibodies to extractable nuclear antigen (ENA) which are RNAse sensitive.[13] Extractable nuclear antigen is prepared from calf thymus cells containing mainly RNP and Sm antigens. This test is performed either by an immunodiffusion technique or by hemagglutination method, to demonstrate antibodies to RNP and Sm antigens. RNP can then be removed from either test system by the addition of RNAse, leaving only Sm antigen if it is present. If a precipitin band remains in the immunodiffusion dish after RNAse is added, this is Sm antigen antibody band, which may be present in SLE and may indicate a poorer prognosis than in those patients with MCTD (Fig 10). The hemagglutination test for RNP and Sm antigen is very similar to the technique employed in the tanned sheep cell test in rheumatoid factor. ENA is added to tanned sheep cells to coat the cells with RNP and Sm antigens. When the patient's serum containing antibodies to ENA is added, agglutination results, indicating a positive test. If after RNAse is added there is no agglutination and the sheep cells fall to the bottom of the tube, this in-

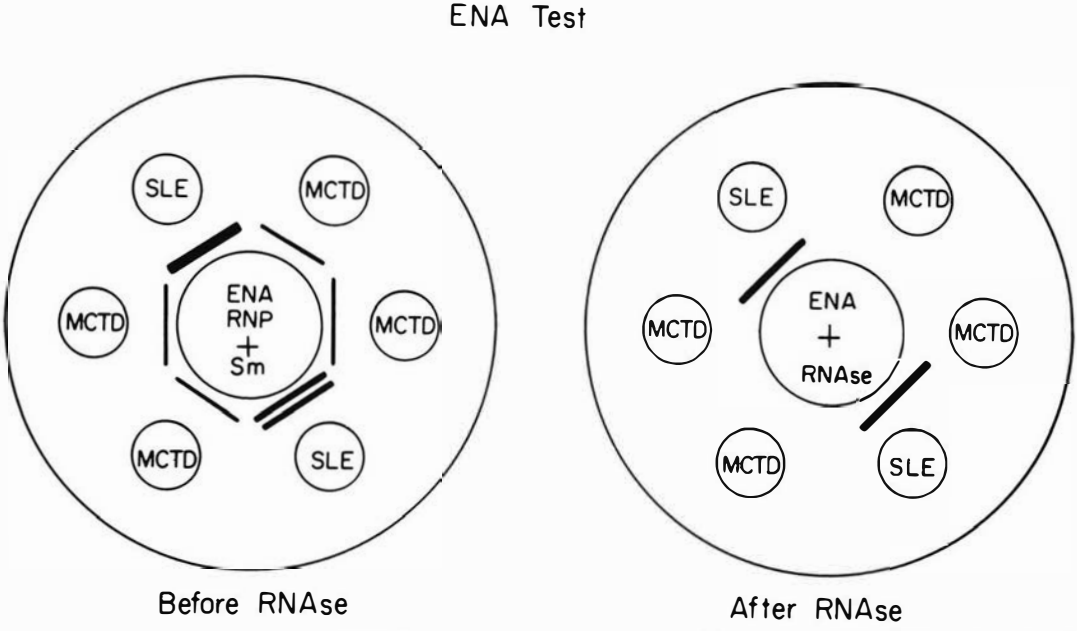

Fig 10—Extractable nuclear antigen test (ENA): Precipitating Antibodies to RNP and Sm Antigens. ENA (RNP and Sm antigens) in center well of agar gel before RNAse show precipitin bands with both MCTD and SLE sera. After RNAse is added to center well in figure on right bands for MCTD, sera are no longer present, leaving only Sm precipitin bands for SLE sera.

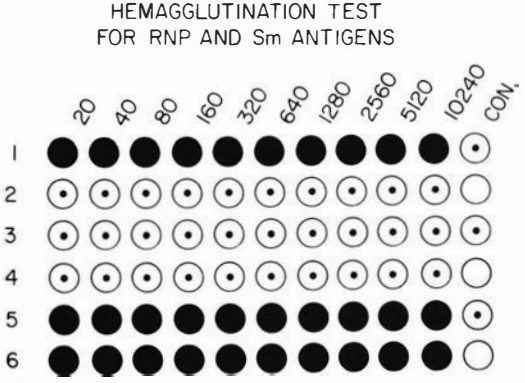

Fig 11—Hemagglutination test for RNP and Sm Antigens. *Row 1* represents a positive ENA agglutination in a titer over 100,000 for MCTD; *Row 2* demonstrates the effect after RNAse has been added, identifying an RNAse-sensitive antibody, and the red cells fall to the bottom of the well, concluding that this serum contains RNP antibodies found in MCTD; *Rows 3* and *4* are sera from a normal individual with negative ENA tests before and after RNAse; *Rows 5* and *6* represent SLE sera which contain antibodies to ENA which are RNAse-resistant, indicating no RNP to be present and Sm antigen accounting for the persistent agglutination. Control sera are in the column on the right.

dicates that only RNP antibodies for MCTD are present. If on the other hand the agglutination remains after RNAse is added, this would indicate other antibodies, such as those to Sm antigen, are present, as is seen in SLE. Titers over 1:10,000 are thought to be significant in the interpretation of this test (Fig 11).

For a diagnosis of mixed connective tissue disease, high titers of hemagglutination antibodies to ENA and no antibodies to ENA after RNAse is added are required. A speckled pattern on fluorescent ANA test is another indication for the diagnosis.

In a group of 100 patients studied by Sharp et al, 74% had RNAse-sensitive ENA by hemagglutination or immunodiffusion techniques, and had mixed connective tissue disease.[13] Of the 26% who were RNAse-resistant ENA patients, a large majority had SLE. This group then was composed of those patients in whom the immunodiffusion and hemagglutination tests showed the presence of antibodies to Sm antigen.

One can conclude, therefore, that those patients who demonstrated antibodies to ENA, which are

RNAse-sensitive (RNP antibody), may have MCTD and are less likely to have renal and CNS involvement and have a better prognosis.

Summary.

1. Rheumatoid factor can be identified in the sera of certain patients with rheumatoid arthritis, is produced in synovial cells, and may have a role in pathogenesis in *some* patients.
2. HL-A B-27 antigen is a genetic marker for development of certain diseases in which spondylitis is a common denominator; its role in pathogenesis is unknown.
3. Systemic lupus erythematosus is an example of antigen-antibody complement complex deposition disease. Different types of antinuclear-antibody tests are associated with different patterns of disease expression.
4. Patients with antibody to the ribonucleoprotein component of extractable nuclear antigen may have mixed connective tissue disease and are less likely to have severe renal and CNS involvement.

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The Polymorphonuclear Neutrophilic Phagocyte

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Patients who have too few functioning mature polymorphonuclear neutrophils frequently develop fatal bacterial or fungal infections despite our best efforts to prevent and treat those infections. Recently new facets of white cell function which enable us to better understand both normal and abnormal states have been found. Several review articles about polymorphonuclear neutrophils (also called neutrophils and granulocytes—the latter term includes eosinophils and basophils) have been published recently[1-7] and the reader is referred to these for more comprehensive coverage of the field.

Development and Deployment of Neutrophils.

Cells that have the potential to develop into the polymorphonuclear neutrophil series or into the monocyte-macrophage series are present in bone marrow. Those destined to be neutrophils go through a sequence of maturation steps lasting about two weeks. The early developing cells have a very rigid cell surface, are poorly motile, and cannot effectively engulf foreign particles. As they develop, granulocytes become progressively more flexible, more able to ingest foreign particles, and more efficient at phagocytosis and killing microbes.

Mature polymorphonuclear neutrophils are constantly released from the marrow into the circulation. Their half-life in the blood is about six to eight hours. After leaving the blood, polymorphonuclear neutrophils migrate to the tissues where they may live for several days before being destroyed. Neutrophils are destroyed by fixed mononuclear phagocytes in such organs as the liver, spleen, lungs, and bone marrow, and others are excreted in the fecal stream. Hundreds of millions of granulocytes enter the bloodstream from the marrow and leave the bloodstream for the tissues each day, but only a small percentage of the body's total number of granulocytes circulates in the blood at any one time. This circulating pool contains between 3% to 5% of the total granulocyte population. The vast majority of mature neutrophils are extravascular in tissues, or waiting to be released from the bone marrow. It is this latter pool that is the source of most of the increased numbers of circulating white cells in patients with acute bacterial infection. There appear to be separate regulators of neutrophil proliferation, maturation, and release into the circulation.

Morphology and Motility.

The mature neutrophil is about 12 to 15 microns in diameter and contains a multilobed nucleus. The characteristic of the cell reponsible for the name "granulocyte" is the presence of multiple granules containing enzymes and preenzymes in the cytoplasm. Other organelles are less conspicuous and very little in the way of ribosomal structure or mitochondria can be seen. There is a large amount of cytoplasmic glycogen which can be utilized as an energy source. Both microtubules and microfilaments can be seen in the cell in special preparations.

Neutrophils are actively motile when in contact with a solid surface: they crawl rather than swim. Motile polymorphonuclear neutrophils look very different from cells seen on a stained blood smear. They usually have a broad front with a thin edge of cytoplasm (the lamellipodia) followed by a slowly ta-

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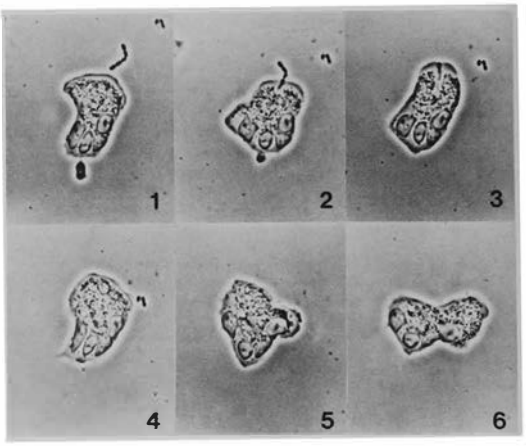
pering, roughly triangular-shaped body and a knob-like tail. Granules are not seen in the leading edge, but granules do move in the cytoplasm of the cell where they seem to travel in roughly-defined channels. There is evidence to support the concept that microtubules and microfilaments are responsible for cell movement.

Phagocytosis.

After mature neutrophils leave the bone marrow and enter the circulation, their mission is to leave the vascular system and migrate to areas where they are needed such as an area of infection or injury. In the first step in this process, called margination, neutrophils circulating through capillaries near the site of injury become sticky and adhere to endothelial cells lining small blood vessels. Anti-inflammatory drugs such as aspirin and prednisone inhibit granulocyte adherence. The phagocytes leave the blood vessels by crawling through the junction between endothelial cells. The neutrophils are then directed towards the site of microbial invasion by chemotaxis, a process in which factors from the region of microbial invasion attract neutrophils towards the greatest concentration of these factors.

After neutrophils have migrated to tissue invaded by microbes, they attempt to ingest susceptible microorganisms and destroy them. The first step is recognition of the microorganisms as foreign to the phagocyte and a target for phagocytosis. With some organisms high titers of specific antibody are required for this recognition while in others the so-called "natural antibody" or low level of antibody usually present in normal serum is enough to stimulate phagocytosis. The antibody itself may promote phagocytosis, but usually the combination of antibody plus complement factors is optimum for promoting phagocytosis. Only immunoglobulins of the IgG class are opsonic.

When a neutrophil engulfs a microbe, the leading edge of the lamellipodia makes initial contact (Figure) and pseudopods are sent out, cupping the microorganism. This cup closes by means of membrane fusion which results in the microorganism being enclosed in a membrane-bound space. This space, called the phagosome, is bounded by the external cell membrane which becomes internalized in the process. The energy for phagocytosis and locomotion is derived largely through anaerobic glycolysis, and cells are able to move and engulf organisms in an anaerobic atmosphere.



Figure—A series of phase contrast photomicrographs of a human polymorphonuclear neutrophil ingesting two clumps of *Streptococcus pyogenes* (original magnification, × 1200). Photomicrograph by James Sullivan and Gerald Mandell.

Leukocyte Bactericidal Activity.

During and after phagocytosis, granules in the cytoplasm move towards phagosomes which contain ingested particles. The contents of the granules are extruded into the phagosome as the granule membrane fuses with the phagosome membrane. The phagosome, now containing lysosomal and other enzymes from the granules, is called the phagolysosome. Degranulation enables these potent enzymes to contact ingested microbes in the phagosome without exposing the cytoplasm of the neutrophil to the possible deleterious effects of the enzymes. Granules "disappear" from the cell during this reaction, hence the term degranulation.

Along with the events described above, changes occur in the metabolism of the neutrophil, the most dramatic of which is a marked stimulation of oxygen consumption. The products of this oxygen consumption are oxidized forms of oxygen such as superoxide anion (O^-_2) and hydrogen peroxide (H_2O_2), both of which possess antibacterial activities. The antibacterial activity of hydrogen peroxide is markedly enhanced by the presence of myeloperoxidase and a halide. In the phagolysosome containing the ingested microbe one finds myeloperoxidase, hydrogen peroxide, and a halide, which in concert can kill the microorganisms. Bacterial death may be the result of a reaction which culminates in oxidation or haloge-

nation, or both, or vital structures on the bacterial surface.

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Immunology and Diseases of the Kidney

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The emphasis of this paper is the review of several aspects of renal disease which have immunologic overtones and clinical relevance. The pathogenesis of several subtypes of glomerulonephritis will be discussed, the immunologic implications of amyloidosis will be noted, and the relation between immune mechanisms and tubulointerstitial disease will be mentioned. The discussion will then be completed by an analysis of the prognosis of the aforementioned renal diseases, and by an attempt to place contemporary therapeutic modalities in a proper perspective.

Before treating each of these subjects, it is worth acknowledging the important contribution which evaluation of renal biopsy tissue has made to our understanding of the relation between immune mechanisms and renal disease. The availability of fresh tissue from biopsies in living patients has allowed adequate evaluation by immunofluorescent microscopy and electron microscopy (EM). Each of these techniques has provided important and complementary information about the nature of fine structural damage induced by immune mechanisms.[1]

Immune Complex Disease.

Proliferative Glomerulonephritis.

Figure 1 is an immunofluorescent stain for IgG in the glomerulus of a patient with acute poststreptococcal glomerulonephritis. A fluorescin-tagged antibody against IgG has been layered over a quick-frozen biopsy specimen and become attached to the IgG. The positive fluorescent lumps are thought to represent deposits of antigen-antibody complexes adjacent to the capillary basement membranes and in the mesangium (the supporting stalk) of the glomerulus. It is assumed that these histologic abnormalities reflect the following series of pathogenic events: an antigenic derivative of the streptococcus has entered the circulation; an antibody response has developed; soluble immune complexes have been formed in a state of antigen excess; the complexes have precipitated in the glomerulus; complement components have been fixed; and the complement cascade activated with resultant production of inflammation and damage to the gomerulus. Under certain circumstances immunofluorescent stains may identify complexes containing IgM and IgA as well as various components of the complement system and fibrinogen.[1]

Figure 2 is a light microscopic view of a glomerulus from another patient with acute poststreptococcal glomerulonephritis. This picture is the light microscopic correlate of the immunofluorescent preparation described above. The features of a diffuse proliferative glomerulonephritis including swelling of the tufts, a marked increase in cellularity (primarily mesangial cells), occlusion of the capillary loops, and an influx of polymorphonuclear leukocytes are present. It is likely that the leukocytes have been attracted by leukotactic factors released by activation of the complement cascade.[1]

Figure 3 is an EM preparation showing part of the ultrastructure of a glomerulus from the biopsy of a patient with poststreptococcal glomerulonephritis. The pathologic findings include fusion of the foot processes of the epithelial cells and deposition of large, electron-dense deposits adjacent to the subendothelial surface of the basement membrane. It is thought that these deposits represent the immune complexes which have been discussed above. Post-

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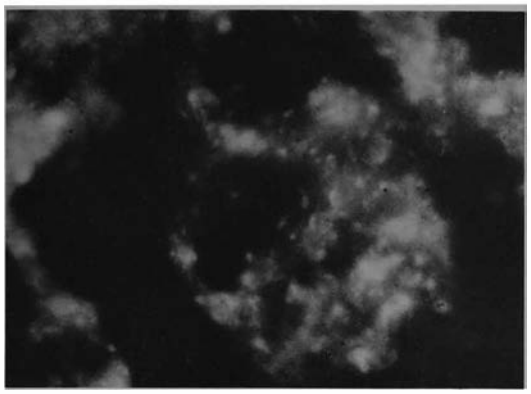

Fig 1—Immunofluorescent microscopic preparation (anti-IgG) of a portion of a glomerulus from a patient with poststreptococcal glomerulonephritis. Note the "lump-bumpy" pattern. (× 1500).

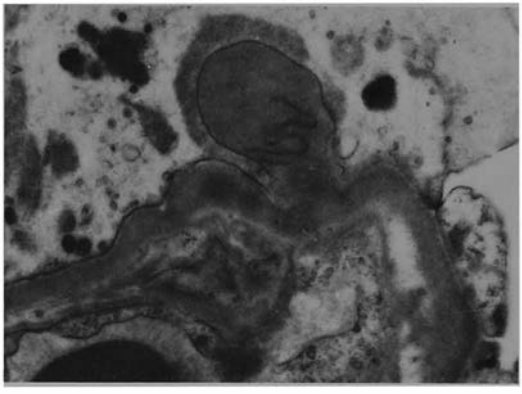

Fig 3—Electron microscopic preparation of a portion of a glomerulus from a patient with acute poststreptococcal glomerulonephritis. Note the large subepithelial electron-dense deposit, "hump" (× 32,000).

streptococcal glomerulonephritis is characteristically associated with large "humplike" deposits in the subepithelial position, but this finding is not absolutely diagnostic because a similar locus of depositon has been noted in other immune-complex-mediated renal diseases such as syphilis with nephritis.[2]

Subsequently, it will become evident that immune complexes may localize on either side of the basement membrane. The reason a deposit may locate in a given site (either subepithelial, subendothelial, or intramembranous) is not entirely defined. Current theory holds that the location of immune-complex deposits may depend upon their physical characteristics, with smaller deposits (formed by combination of antigen with low affinity antibody) localizing in a subepithelial site and large complexes localizing subendothelially.[1] There is also evidence to suggest that complement-binding sites may be present in the region of the epithelial cell foot processes.[3] Complexes which have fixed complement prior to traversing the basement membrane may be snared by attachment of complement with these receptors.

Figure 4 is the light microscopic view of two glomeruli from a patient with a staphylococcal albus

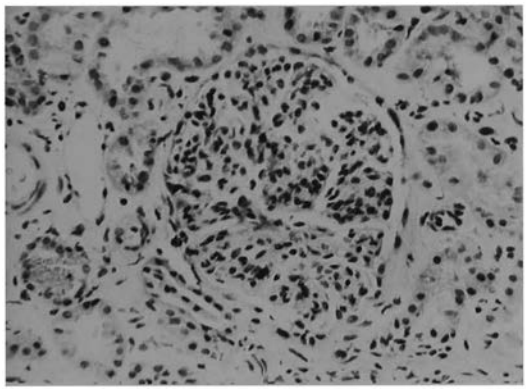

Fig 2—Light microscopic preparation (H & E) of a portion of a renal biopsy from a patient with diffuse proliferative glomerulonephritis following a streptococcal infection (× 200).

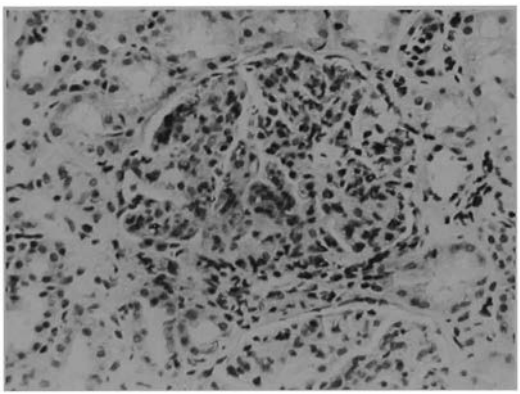

Fig 4—Light microscopic preparation (H & E) of a portion of a renal biopsy from a patient with "shunt nephritis" secondary to staphylococcal infection. Note that glomerulus in the center and the portion of the glomerulus at the lower margin both show proliferative changes (× 200).

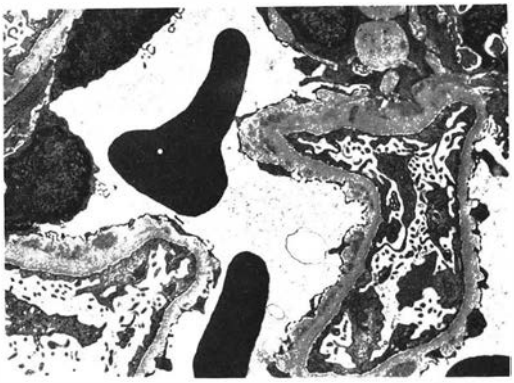

Fig 5—Electron microscopic preparation of a portion of a glomerulus from a patient with systemic lupus erythematosus and a diffuse proliferative lesion by light microscopy. Note the large subendothelial deposits (× 10,000).

infection of a ventriculojugular shunt which had been made because of hydrocephalus. The lesion is a diffuse proliferative glomerulonephritis and could not be distinguished from the poststreptococcal lesion by conventional light microscopic or immunofluorescent studies. Figure 5 is an EM study showing a large subendothelial deposit from a patient with lupus erythematosus and proliferative glomerulonephritis by light microscopy. The subendothelial region is the favored site of deposition in lupus.[1] Figure 6 shows the light microscopic appearance of a glomerulus from a patient with focal proliferative glomerulonephritis. Note that segments of the glomerular tuft appear to have normal cellularity and that the inflammatory reaction is less severe than in the cases noted earlier. Immunofluorescent and electron microscopic studies would show the immune deposits to be less numerous and to have more of a mesangial location than in the diffuse proliferative lesion. IgA deposition is seen with greater frequency in the mesangium in focal lesions.[1]

After a review of the first six figures it is clear that the classic light microscopic picture of proliferative glomerulonephritis, either diffuse or focal, reflects a disorder of immune-complex deposition in the region of the glomerular capillary basement membrane and mesangium. From observation of the pathologic material it is also easy to envision that the inflammatory process would lead to leakage of albumin, red blood cells, and immunoproteins into the urine with development of an "active" urine sediment. The mediators of inflammation in proliferative lesions are not well understood but are thought to be released by activation of the terminal portion of the complement cascade.[1] Serum levels of early-reacting complement components and C_3 are frequently, but not invariably, reduced in patients with immune-complex-mediated proliferative lesions. The antigenic substances which may be involved in this type of lesion are numerous and will be discussed below.

Membranous Glomerulonephritis.

Another histologic pattern of renal involvement that is almost certainly mediated by glomerular immune-complex deposition is membranous glomerulonephritis (Fig 7). Note that the only abnormality is marked thickening of the capillary basement mem-

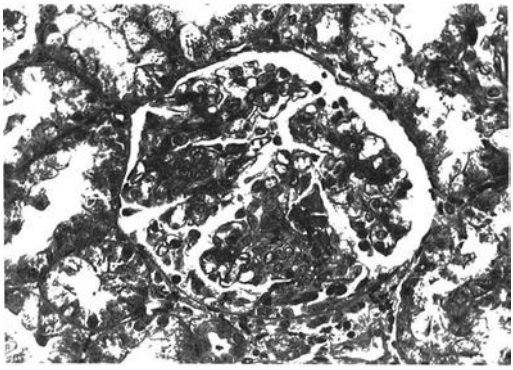

Fig 6—Light microscopic preparation (H & E) of a portion of a renal biopsy from a patient with systemic lupus erythematosus showing a focal proliferative lesion (× 400).

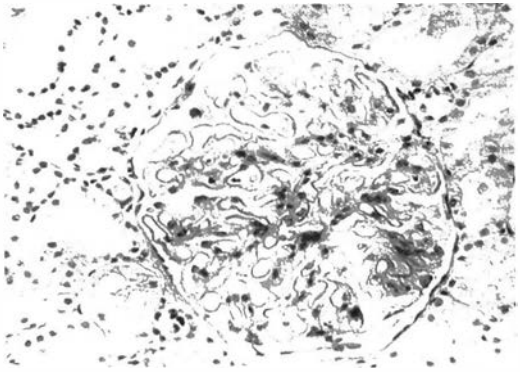

Fig 7—Light microscopic preparation (PAS) of a portion of a glomerulus from a patient with membranous glomerulonephritis (× 200).

brane. Figure 8 demonstrates that the cause of the basement membrane thickening is the presence of numerous dense deposits along the epithelial border of the basement membrane with projections of basement membranelike material interposed between the deposits. Fusion of the epithelial cell foot processes adds to the breadth of the capillary wall and probably has been induced by the marked albumin leak which most of these patients experience. Immunofluorescent studies show a fine granular deposition of IgG and IgM as would be expected from the location of the deposits on EM.[1] On occasion, complement components also may be identified in the glomerular capillaries, but this occurs with much less frequency and in a more scant distribution than in proliferative lesions. Serum complement levels are usually normal in membranous glomerulonephritis. The absence of an inflammatory response may relate to the presence of immune complexes which fix complement poorly, or to an impotent complement system. As might be expected, patients with the membranous lesion usually develop a nephrotic syndrome and tend to have a less "active" sediment than those with a proliferative process.

Antigens Associated with Immune Complex Disease.

There are a large number of disorders in which antigen-antibody complex deposition is recognized or suspected as being the cause of renal disease.[1] Exogenous antigens, particularly drugs and foreign proteins, may induce an immune-complex glomerulonephritis with the appearance of either a proliferative or a membranous lesion. A number of bacterial organisms including the streptococcus, staphylococcus, pneumococcus, and treponema pallidum have induced a complex-mediated nephritis which is usually proliferative in pattern. Plasmodium malariae may cause either a proliferative or membranous nephritis. It is suspected that numerous viral agents may incite immune-complex-mediated renal disease. Both hepatitis-B virus and the Barr-Epstein virus have been clearly identified as providing the antigenic stimulus for an immune-complex-mediated disorder. The former has been incriminated as inducing proliferative and membranous lesions as well as a generalized arteritis.

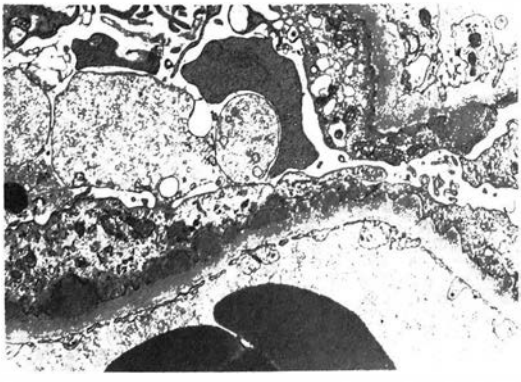

Fig 8—Electron microscopic preparation of a portion of a glomerulus from a patient with membranous glomerulonephritis. Note the numerous subepithelial deposits. (× 15,800).

Perhaps the best defined of all immune-complex-mediated renal diseases is that associated with systemic lupus erythematosus. It is clear that in this disorder endogenous cellular antigen (DNA, RNA, and numerous derivative substances) provides an inexhaustible source of antigen for complex formation. Diffuse proliferative, focal proliferative, and membranous nephropathy have been identified in patients with systemic lupus.

Recently, considerable excitement has been generated by the discovery of other endogenously-produced antigens which may cause renal disease in man. A derivative of tubular brush border has been identified as the antigenic component of an immune-complex-induced membranous glomerulonephritis in patients with sickle cell anemia.[4] The nephrotic syndrome secondary to a membranous lesion has been recognized in some patients with solid tumors of the lung and colon; in a patient with the latter neoplasm carcinoembryonic antigen has been identified as part of the complex on the basement membrane.[5] Immune-complex glomerulonephritis may be seen with some frequency in patients with cryoglobulinemia, particularly those in whom there is a mixed IgG-IgM cryoglobulin with rheumatoid factor activity.[6]

After reviewing all of the known causes of immune-complex-mediated renal disease one is left with an unrecognized antigen as the stimulus for the disease process in most cases of the idiopathic nephrotic syndrome. This includes patients with focal proliferative, diffuse proliferative, or membranous lesions. The offending antigen is also unknown in such syndromes as Wegener's granulomatosis and Henoch-Schönlein purpura. Within the next few years the inciting antigens will be identified in many of these disorders and may well prove to be viral agents.

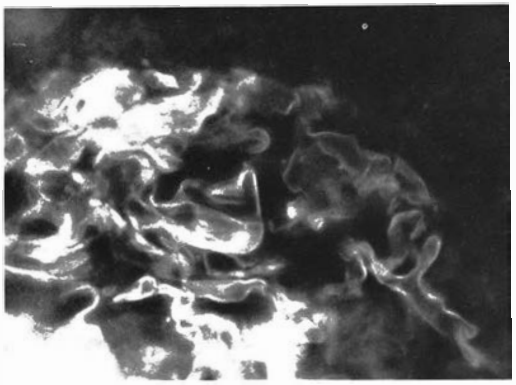

Fig 9—Immunofluorescent preparation (IgG) from a portion of a biopsy of a patient with antibasement membrane antibody disease. Note the linear pattern of fluorescence (× 1500).

Antibasement Membrane Antibody Disease.

Figure 9 is an immunofluorescent stain showing homogeneous linear deposition of IgG along the basement membrane of the glomerulus. This pattern is thought to represent the attachment of antibasement membrane antibody to some antigenic component in the basement membrane. The antibody is usually IgG although linear deposition of IgM and complement have also been described in some cases. In many patients, circulating antibody can be demonstrated by allowing the patient's serum to react with sections of normal human kidney. The mechanism whereby deposition of antibasement membrane antibody leads to renal damage is uncertain, but that severe damage can be induced is amply demonstrated by Figure 10 which shows a proliferative lesion of both the mesangial and epithelial cells. Proliferation of the latter with crescent formation is apparently induced by the leakage of large molecular weight fibrin precursors into Bowman's space through tears in the basement membrane.[1]

An occasional epithelial crescent can be found in virtually any type of renal disease, but involvement of essentially the entire glomerular population is seen in two circumstances: rapidly progressive glomerulonephritis and Goodpasture's syndrome. Many patients with the clinical picture of idiopathic, rapidly progressive glomerulonephritis and most patients with Goodpasture's syndrome (lung hemorrhage and nephritis) will display evidence of antibasement membrane antibody as the cause of renal damage. The offending antibody in Goodpasture's syndrome cross-reacts with pulmonary basement membrane and is thought to induce the pulmonary as well as the renal disease. The stimulus for production of antibasement membrane antibody is uncertain, but damage to pulmonary or glomerular basement membrane by viral agents or chemical irritants with uncovering of hidden antigenic sites has been suggested as a mechanism.[1] As might be expected, patients with antibasement membrane antibody disease and crescent formation frequently have very "active" urine sediments and may be nephrotic.

Glomerulonephritis and Activation of the Alternate Complement Pathway (Membranoproliferative Glomerulonephritis).

In general terms, when the complement system is activated in the disorders discussed above, it is probably brought about by the classic pathway ($C_1 \rightarrow C_4 \rightarrow C_2 \rightarrow C_3$). Recently, however, evidence has begun to accumulate suggesting that activation of the latter part of the complement cascade via the so-called "alternate pathway" may be of importance in inducing renal damage.[1] Activation of the "alternate pathway" has been noted in some cases of poststreptococcal glomerulonephritis, but the most provocative evidence for a role of this pathway has been observed in patients with the pattern of membranoproliferative or mesangiosclerotic glomerulonephritis.

West and his associates originally described a group of children with heavy proteinuria; "active" urine sediments; low circulating C_3 levels; light mi-

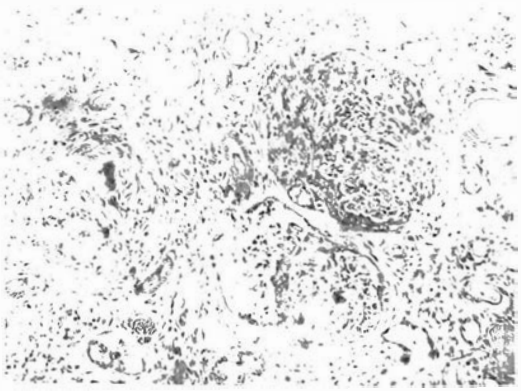

Fig 10—Light microscopic preparation (H & E) from the renal biopsy of a patient with antibasement membrane antibody disease. Note the marked crescent formation and interstitial inflammatory reaction (× 80).

croscopic evidence of cellular proliferation, increased mesangial matrix, and a tendency to lobulation of the glomerular tufts; glomerular C_3 deposition with little or no accompanying IgG or IgM by immunofluorescence; and a circulating activator of C_3 (C_3 nephritic factor).[8] The patients were considered to be a unique group and designated as having membranoproliferative glomerulonephritis with hypocomplementemia. Subsequently, it has been recognized that patients with membranoproliferative lesions are not a homogeneous group; and they have now been subdivided into two groups, primarily on the basis of EM features. Type I patients show evidence of splitting and reduplication of the basement membrane and have electron dense deposits. The activation of early complement components and the infrequent demonstration of circulating C_3 nephritic factor in this group suggest the presence of a classic immune-complex-mediated disease. Type II patients, on the other hand, show a marked homogeneous increase in density of the glomerular capillary basement membrane without evidence of deposits (dense deposit disease).[1] Circulating C_3 nephritic factor is usually present and early reactive complement components are normal. The nature of the dense transformation of the basement membrane is uncertain and the pathogenesis of this disorder remains to be defined.

Lipoid Nephrosis of Childhood (Nil Disease).

Another disorder which classically has been considered with the glomerulonephritides is lipoid nephrosis of childhood or nil disease. This is the most common cause of the nephrotic syndrome in childhood. The only recognizable histologic abnormality in patients with this disturbance is fusion of the foot processes recognizable on EM (Fig 11). The etiology of this disorder is uncertain, but renewed interest in the possibility of its being an immunologic disease has been kindled by the discovery of a similar lesion in several patients with Hodgkin's disease who have the nephrotic syndrome. Since current thought suggests that Hodgkin's disease may be a T-cell disturbance, it has been suggested that nil disease reflects a disturbance of cellular immunity.[9]

Focal Segmental Sclerosis.

A subgroup of children with the idiopathic nephrotic syndrome who do not respond well to the therapeutic agents mentioned below has been described recently. This group frequently has mild microscopic hematuria as opposed to the children with typical nil disease who show no "activity" of the urinary sediment. Segmental sclerosis beginning in the juxtamedullary glomeruli and progressing to become a diffuse generalized involvement with ultimate global sclerosis has been identified in these children.

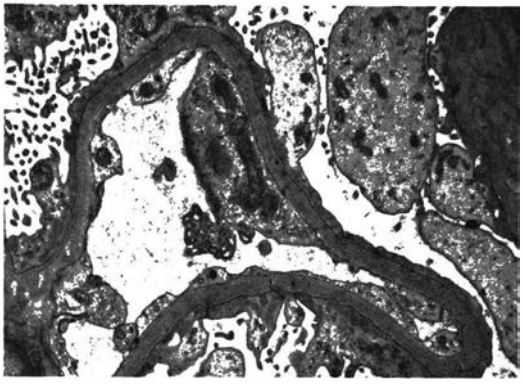

Fig 11—Electron microscopic preparation of a portion of a renal biopsy from a patient with "nil disease." Note the normal appearing basement membrane, the absence of deposits, and the presence of fused foot processes (× 14,000).

Amyloidosis.

Amyloidosis of the kidney is an interesting cause of the nephrotic syndrome which, on clinical grounds, may be confused with the glomerular diseases previously mentioned and which has a close relationship to the body's immune systems. On light microscopy the glomerular mesangium may appear infiltrated with a homogeneous material and the basement membrane may be thickened. Such a pattern may be confused with diabetic nephropathy or a late stage of immune-complex-mediated nephropathy which has led to significant sclerosis; staining with Congo red and viewing the sections under polarized light will demonstrate the typical apple-green birefringence if amyloid fibrils are present, however. The amyloid fibril also gives a characteristic appearance by EM as demonstrated in Figure 12.

Exciting recent work has provided a clearer understanding of the nature of amyloid. The amyloid fibrils seen in patients with B-cell dyscrasias, either multiple myeloma or primary amyloidosis, are thought to be composed of light-chain derivatives of the paraprotein produced by the abnormal B cells.[10] An entirely unique protein (AA protein) has been identified as composing the amyloid of patients with secondary amyloidosis related to chronic infection or

familial Mediterranean fever.[10] The reason that these different proteins have similar refractive properties and give a similar appearance on EM relates to the fact that they share the same beta-pleated arrangement of their amino acid components.

Tubulointerstitial Disease.

Investigation of the immunologic aspects of tubulointerstitial disease has been overshadowed by the tidal wave of studies evaluating the mechanisms of immunologic glomerular damage. Interest in the interstitium has been rekindled, however, by the discovery of an association between the ingestion of certain drugs and the development of interstitial nephritis. It is now evident that a molecular moiety of several members of the penicillin family may act as a hapten, combine with an endogenous protein to make a complete antigen, stimulate an antibody response, and ultimately result in precipitation of immune complexes in the region of the tubular basement membranes with incitement of a diffuse interstitial inflammatory reaction.[11] Autoantibodies to tubular basement membrane have also been demonstrated and incriminated as the cause of interstitial infiltration in lupus erythematosus, the transplanted kidney, and the crescentic disease of Goodpasture's syndrome and rapidly progressive glomerulonephritis (Fig 10).[1] Studies in animals have suggested that chronic pyelonephritis may be perpetuated by cellular immune mechanisms originally activated by the release of antigenic substances from tissues damaged by invading pathogenic bacteria.[1]

Prognosis and Treatment.

Having reviewed the current thoughts about the pathogenesis and histologic appearance of immunologically related renal disease, we ought now to attempt to apply this information in a fashion that will be beneficial to a given patient. Such application is practical in two major areas, prognosis and treatment. The importance of a renal biopsy in obtaining tissue for evaluation is obvious. Its importance in obtaining practical prognostic and therapeutic information which will help the patient is variable. For example, a renal biopsy is not likely to be helpful in the management of the patient with obvious interstitial disease as manifested by leukocytes and a relatively scant amount of albumin in the urine. If the disease is drug induced, it will probably resolve spontaneously with discontinuation of the offending agent.

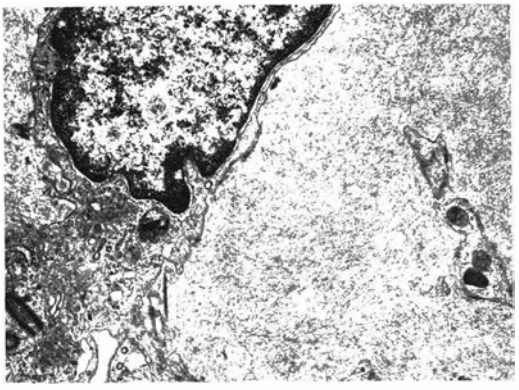

Fig 12—Electron microscopic preparation of a portion of a glomerulus showing amyloid fibrils (× 24,000).

It is among patients with an unexplained nephrotic syndrome that evaluation of an adequate renal biopsy is most helpful.

Prognosis.

Patients with focal proliferative glomerulonephritis, lipoid nephrosis, and membranous glomerulonephritis have a relatively good prognosis either treated or untreated. Lupus patients with a focal proliferative lesion may live for years without showing evidence of deterioration in renal function. Many children with nil disease undergo a spontaneous remission and when deterioration in function occurs, it usually progresses very slowly. Patients with drug-related or tumor-related membranous nephropathy may have remission of their disease with removal of the offending antigen; those with an idiopathic membranous lesion, or lupus and a membranous lesion, may spontaneously remit or remain nephrotic with an unchanged creatinine clearance for years.[1]

On the other hand, patients with diffuse proliferative lesions unrelated to a specific infecting organism, particularly those with lupus erythematosus, have a poor prognosis. Most patients with antibasement membrane antibody disease and marked crescent formation will reach the terminal stage within two years.

Patients with membranoproliferative glomerulonephritis tend to have an intermediate prognosis with most showing progressive deterioration in renal function to its terminal stage in 5 to 12 years.[1] Amyloid patients and patients with segmental sclerosis also demonstrate an intermediate prognosis.

As knowledge accumulates, we may feel obliged

to perform a biopsy on every patient with chronic renal disease who is progressing toward its terminal stage in order to determine his or her ultimate candidacy as a transplant recipient. Antibasement membrane antibody disease, membranoproliferative glomerulonephritis, and focal segmental sclerosis have been recognized as recurring in grafted kidneys, ultimately leading to their failure. Indeed, it is now considered inappropriate to transplant a patient with antibasement membrane antibody disease if circulating antibodies can be demonstrated in his or her serum.[1]

Treatment.

Much has been written about the treatment of immunologically related renal diseases. Unfortunately, except for the management of two disorders, nil disease and Wegener's granulomatosis, there is little agreement and much bias about specific treatment. Diuretics are of great symptomatic value in edematous states but have no effect on basic pathogenic mechanisms. The agents which have been used as possible inhibitors of immune mechanisms include corticosteroids, antimetabolites, and alkylating agents. Each of these drugs has significant toxicity, particularly when used over a prolonged period, and their modes of action remain uncertain. The natural history of many of the immunologically related renal diseases is variable and capricious. Consequently, controlled studies, involving numerous patients and extending over a long period of time, are needed to establish the efficacy of any drug regimen. These studies have not been done. Outlined below is a personal assessment of the state of the art of immunotherapy in renal disease.

As already mentioned there seems to be uniform agreement that therapy is effective in those patients with nil disease and those with Wegener's granulomatosis. In lipoid nephrosis the administration of corticosteroids will enhance the rate of remission, and the duration of remission after relapse can be prolonged by supplementing the steroids with cyclophosphamide or chlorambucil. Nil disease in Hodgkin's disease will subside with treatment of the lymphoma. Cyclophosphamide is effective in producing a sustained remission in the renal disease of Wegener's granulomatosis, a disorder which was formerly uniformly lethal.[12]

Treatment in a patient with immune-complex-mediated diffuse proliferative or focal glomerulonephritis is most effective if it can be directed at eradication of a recognizable inciting antigen. Obviously, this is most successful if the antigen is derived from an infectious agent such as a staphylococcus or a spirochete. If the antigen cannot be removed as in lupus erythematosus, one must carefully weigh the potential benefits of therapy against the possibility of significant drug toxicity. It is my impression that most authorities would tend not to treat a focal proliferative lesion in lupus but would be aggressive in the management of a patient with a diffuse proliferative lesion. In the latter case, one might use a combination of steroids and either azothioprine or cyclophosphamide in doses sufficient to return serologic indicators of disease activity such as C_3 levels or anti-DNA antibody levels to normal.[13]

The management of membranous glomerulonephritis of either the idiopathic type or that associated with lupus erythematosus is equally perplexing. Controlled studies done in Great Britain in the 60s suggested that no therapy was effective in the idiopathic membranous lesion.[14] However, recent uncontrolled retrospective analysis[15,16] and an ongoing American cooperative study[17] suggest that prolonged steroid administration may be helpful.

Therapy for patients with crescentic disease has included steroids, azothioprine, cyclophosphamide, and anticoagulation agents. Isolated cases treated with these agents have shown good responses, but there have been no controlled studies to substantiate this fact. Steroids have been advocated as being of value in membranoproliferative lesions,[18] but here again there have been no comparisons with a comparable untreated population.

There seems to be no specific treatment for amyloidosis. However, amyloid deposition may be inhibited by treatment of an underlying myelomatous state or by correction of a smoldering infectious process.[1] Success has been reported recently in reducing proteinuria in amyloid disease secondary to familial Mediterranean fever by the administration of colchicine.[10]

Summary.

The relation between the immunologic systems and renal disease has been briefly reviewed and several pathogenic immunologic mechanisms have been correlated with the histologic pictures which they produce; this information has then been related to contemporary thoughts about prognosis and therapy. Much has been learned, but there is obviously much

more to be done, particularly in the areas of prevention and treatment.

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Cancer: The Great Challenge for Immunology

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The existence of immunity to cancer was postulated by the eminent scientists who helped to establish the discipline of immunology. In 1907 Clowes suggested that human resistance to cancer resulted from what we today call "immune surveillance."[1,2] During the ensuing 70 years the results obtained from experimental animal cancers and human cancers have greatly influenced the palatability of tumor-immunity theories.[3,4] Early optimism that immunity to cancer could be specifically induced waned and almost disappeared when it was demonstrated that the rejection of cancer transplants resulted from transplantation immunity and not tumor immunity. A sustained wave of enthusiasm for immunity to cancer appeared after demonstrations that inbred animals could be immunized to cancers arising in the inbred strain.[5,6]

The objective of this paper is to try to reexamine many aspects of cancer immunology and to shift the emphasis currently placed on some of these aspects into other areas with greater potential for clinical application. This is not meant to be one of the numerous reviews of cancer immunology but rather a balanced presentation of alternative viewpoints which will ultimately tilt toward my viewpoint.

The plan for the paper is as follows: (1) the types of contributions immunology has made to mankind will be briefly reviewed; (2) a general theory describing cancer immunity with some supporting evidence will be presented not once but twice; (3) finally the current status of immunotherapy of human cancer will be briefly mentioned.

Contributions of Immunology.

A review of the major contributions of immunology to humanity should offer a preview of what can reasonably be expected from future contributions of this field to the understanding and control of cancer. The trademark of immunology is prevention of disease by immunization. With the discovery of antibiotics and their use in tissue cultures, a fresh attack upon many viral infections became possible. In the past two decades, the cultivation of viruses in vitro has resulted in the elimination of epidemics of poliomyelitis; infection with measles virus is less common. Where the human is the sole host and reservoir of an infection, immunization may lead to the eradication of a disease. This appears to be the attainable goal in smallpox where we are at the threshold of its eradication by intensive immunization and epidemiologic field work.

While the trademark is immunization, the work horse of immunology is serology. Its use in diagnosis and blood banking alone are of critical importance to the functioning of our hospitals. A strike of all technicians doing serologic tests would paralyze our health care system. In addition to its diagnostic contributions, immunology provides an important understanding of the pathogenesis of disease.

A relatively new but potentially major contribution is in predicting susceptibility to disease. The association of certain transplantation antigens with specific disease states may be the forerunner of serologic identification of disease-risk factors.

When one turns to the role of immunology in therapy, the work seems harder and the results hardly optimal. This view is not meant to belittle the value of replacement therapy in certain immune deficiency diseases, or of immunosuppressive therapy in preventing transplant rejection; rather it is intended to point out that cancer immunology is much, much more than immunotherapy.

General Features of Immunity to Cancer.

All cancer immunology is inextricably linked to the existence of an antigenic difference between the

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cancer cell and its closest normal "relative" cells. Without such a difference, immunology has no entry into the cancer field.

The transformation of a normal cell to a cancer cell is probably accompanied by an antigenic change. This transformation may occur frequently in all of us. With a properly functioning immune system, the antigenic change or changes in the cancer cells are recognized and following recognition an effective anticancer cell immune response destroys the malignant cells. Appearance of clinical cancer is thus considered to be a prima facie case of a failure of normally operating immunologic mechanisms.

Evidence Supporting Immune Surveillance.

Origins for cancer antigens are not hard to find. Oncogenic viruses are obvious sources of extraneous antigenic material incorporated into cancer cells. While no human oncogenic virus has been clearly identified, several viruses are viewed with suspicion. Chemical compounds play an important role in the initiation of human cancer, and for many years these carcinogens included many compounds that are also mutagens. Recently a bacterial test for detecting chemical mutagens has shown that almost every known chemical carcinogen is either a mutagenic agent or is metabolized to a mutagen.[7] Chemical carcinogen-induced changes in the bases of deoxyribonucleic acid (DNA) can result in the synthesis of abnormal, that is, antigenic, proteins. Similarly, physical agents such as ultraviolet and x-irradiation are also known carcinogens and mutagens.

Amongst the wide variety of human cancers, an impressive list of cancer-associated "time and place" antigens have been detected.[8-10] The carcinoembryonic antigen of the gastrointestinal tract, alpha-fetoprotein, chorionic gonadotropin, antidiuretic hormone, and parathormone are examples of normal products made by cancers that are either abnormal for postnatal life or for that type of cell.

As we move to consider the evidence for the existence of immune cancer-rejection systems, we need to rely on negative observations. It is impossible to demonstrate that we are cancer-free as a result of active recognition and destruction of small clones of cancer cells, but strong circumstantial evidence points to such immune mechanisms. Patients who are recipients of organ transplants have a high risk of subsequently developing a malignant disease[11,12]; these patients are estimated to be at least 25 times more likely to develop cancer than the normal population.

An additional group of patients at high risk for developing cancer are those individuals with immunodeficiency diseases. From 5% to 10% of patients with sex-linked agammaglobulinemia, combined immunodeficiency disease, Wiskott-Aldrich, or ataxia telangiectasia will develop clinical malignant disease. Further but less definite indications that we are protected by immune mechanisms include the observations that chemical carcinogens may be immunosuppressive,[14] the claims of cutaneous anergy in patients with neoplastic disease,[15-17] and the frequently stated view that patients with cancer have an increased susceptibility to infection.[18]

The last element to be considered in the construction of an immunologic lattice for the containment of cancer is the alteration of the course of cancer by immunologic methods—immunotherapy. Attempts to stimulate a specific immune response[19] and to stimulate the entire immune response by agents like bacille Calmette Guérin (BCG)[20] have been extensively performed. In 1971, a comprehensive review was published by Yashpie,[21] and the report of a conference entitled, "Immunotherapy of Cancer: Present Status of Trials in Man," held in Washington in October, 1976, is to be published.

How effective is immunotherapy for human cancer? It is important to realize that the concept of the "proof of the pudding is in the eating" is as much determined by how hungry one is as by the quality of the pudding. Rather than enthuse about immunotherapy, I prefer to accept its present meagre results as a challenge to reexamine our entire position. I will also consider immunology with respect to prevention, pathogenesis, early diagnosis, treatment monitoring aids, and immunotherapy.

Prevention of Human Cancer by Vaccines.

An extensive review of the possibilities in this area was recently published.[23] At least two major obstacles need to be overcome before vaccines for human cancer become a reality. First and foremost the link between a human virus and the cancer it causes needs to be firmly established. Then the virus can be developed into a vaccine—living, killed, or subunit. The second problem is to determine who should receive the vaccine. Since the incidence of any one kind of carcinoma is relatively low, methods are needed to identify the high-risk groups. Where the prevalence of a carcinoma may be 5 to 10 persons per 100,000, it would be unacceptable to try to immunize the whole population.

Where a viral-associated neoplasm behaves like

a communicable infectious disease, a vaccine could be very helpful. Such a situation exists in the poultry industry. A DNA herpes-like virus (Marek's disease virus) is manufactured into a fully infectious form in the feather follicle of the chicken. In addition this virus spreads within the chicken and causes a fatal lymphoreticular disease; it also spreads amongst chickens and can wipe out a flock. An effective vaccine has been prepared from an apparently harmless herpes virus of turkeys. This vaccine protects the chickens against Marek's disease.[23]

The Pathogenesis of Cancer.

Immunologists searching for human cancer antigens have made an astounding, although largely ignored, contribution to our understanding of the pathogenesis of cancer. Despite years of search by numerous competent investigators, a cancer-specific antigen has not been isolated for any human cancer. Although the search for cancer-specific antigens is too important to be abandoned, the possibility that specific cancer antigens do not exist must be faced. Instead of cancer-specific antigens, cancer-associated antigens have been found. Some of these antigens are considered time antigens. A cancer cell makes fetal alkaline phosphatase, or a fetal pyruvate kinase isozyme, or embryonic antigens, or structures such as alpha-fetoproteins; place antigens also are made. Thus a variety of normal hormones are made by malignant cells derived from cells that have ceased making these products. Frequently these hormones produce symptoms in the patient, a paraneoplastic syndrome. Were we to have the full catalog of normal gene products made from conception to maturity, it is possible that a time or place antigen or both could be associated with every human cancer. The finding of time and place cancer-associated antigens instead of cancer-specific antigens fits in with an intriguing new concept of the pathogenesis of cancer,[24] which as its essential feature regards cancer as a programming error. Carcinogenesis is not a mutation to new structures but rather a reactivation of genetic programs that were terminated a long time ago. In this view viruses, chemicals, and physical agents act by going into the "old book" section of the cell's DNA library and activating something long dormant.

It is possible to estimate the percent of the informational DNA that is being actively transcribed by cells. There is no difference in the amount of DNA active in the blastula phase, the gastrula phase, or the adult cells. About 3% of the DNA is being used, but the 3% used in the gastrula phase cells is not identical to the 3% used by the blastula cells. Thus normal development consists of the orderly and sequential production and elimination of portions of the DNA program.

Can programs be initiated? Dr. Ruddy referred to androgen treatment of hereditary angioedema. The administration of an androgen leads to synthesis of a significant amount of a protein necessary to inhibit spontaneous activation of the complement system; other examples exist, perhaps the best being the reactivation of the information locked up within a cell nucleus as reported by Gurdon.[25] Transplantation of organelles produced striking results when the nucleus of a fertilized frog ovum was removed and replaced by the nucleus of a mature frog muscle cell. The microsurgically treated cell was then restored to its proper environment, and development of the ovum resulted in the formation of a tadpole. All the information for this development was uncovered in an orderly fashion from the mature nucleus of a differentiated cell. Similar results have been obtained when the nucleus from a mature frog lymphocyte was transplanted into an enucleated fertilized frog ovum.

Our society seems to have more difficulty in formulating the correct questions than it does in providing the answers to these questions. The finding of several cancer-associated antigens emphasizes that cancer immunologists must continue to examine serologically the early stages of development with the objective of identifying additional tumor-associated antigens that in turn may be critical in establishing valid early diagnostic tests for cancer.

The Nature of the Immune Defect in Cancer.

The failure to demonstrate an effective immunotherapeutic method requires that the defects in the immune surveillance and rejection system be examined again with respect to cancer.

Does the patient who develops a carcinoma of the lung, or breast, or stomach or other organs have a defect that is applicable to the recognition and reaction to many antigens or is the defect confined to the antigen or antigens associated with that particular cancer? This is not a trivial question since the direction for future immunotherapy depends on the answer. Arguments in favor of a broad defect are the high incidence of neoplasm in transplant patients and in those with immune deficiency diseases, but the interpretation of this evidence is not decisive. Cytotoxic immunotherapy is not exclusively immunosuppressive. It may interfere with DNA repair mech-

anisms which if unchecked could cause malignancy as seen in xeroderma pigmentosa.[26] These patients have a very high incidence of neoplasms of the skin, and severe impairment of the ability to repair the damage in DNA caused by ultraviolet irradiation. Many of the drugs used in immunosuppression may also interfere with DNA repair mechanisms.

The evidence suggesting that there is no broad immune defect in cancer patients is drawn from the incidence of infection in patients with solid neoplasms. Since the earliest days of immunology, infection has pointed to the areas where immune defects exist, and it is unusual to see clinically significant immune defects without concomitant frequent infection. Indeed the defects may be so subtle, as in sickle cell disease, that increased susceptibility to infection is recognized long before the nature of the immune defect is discovered.

Contrary to general opinion, infection is not a common problem in the patient with solid cancers, although infection certainly occurs when large masses obstruct a passageway or become necrotic. If extensive chemotherapy renders the patient granulocytopenic, or if large doses of steroids are given, infection occurs, but under other circumstances, infection in a non-terminal cancer patient is rare. Accounts of infection in cancer patients are predominantly those of patients with leukemia, lymphoma, and myeloma. Of 93 patients with aspergillosis, only 14 had solid tumors.[27] Of these, 11 were receiving steroids and nine were receiving cytotoxic drugs. Another recent report[28] shows that 31 of 35 patients treated for infection with sulfamethoxazole-trimethoprim had hematologic malignancies; so it goes with all reports of infection in cancer patients.

It appears to me unlikely that the overwhelming majority of patients with solid tumors have a large blind spot in their immune system. Skin testing for anergy, counting T and B lymphocytes, and stimulating lymphocytes with mitogens can probably be safely discontinued or replaced by looking for the real defect in the immune system in cancer patients.

This leads to the second question. How does an antigenic cancer escape detection? The answer to this question is beset with technical difficulties. The reports of two workshops[29,30] designed to evaluate the results of in vitro cytotoxicity tests for cancer cells are gloomy. More emphasis needs to be placed upon technical improvements in the culturing of cancer cells and in determining their in vitro susceptibility to antibody and to lymphocytes and macrophages.

Our understanding of this area is intimately tied to our efforts in human cancer immunotherapy. An outline of how a cancer breaks through or may break through is of value even though it is purely speculative. Early studies in malignant melanoma[31] stressed the importance of humoral antibodies. Patients with localized melanoma were reported to have antibody which reacted with melanoma cells while patients with disseminated melanoma generally lacked these antibodies. Using in vitro techniques, the Hellstroms demonstrated a more complex Trojan Horse type of immunologic arrangement[32] in which lymphocytes from a cancer patient could destroy in vitro cancer cells removed from that patient. This cellular immune reaction could be inhibited by antibody present in the serum of that cancer patient and from these observations a dual immune system was formulated—antibody could protect the cancer, and cellular immunity could destroy the cancer. Further modifications have been made in both the serum and cellular aspects, but the basic premise remains that the destruction or growth of a cancer depends upon the relative strengths of two types of immune reactions. This point should be returned to in considering the results of immunotherapy.

Immunology and Early Diagnosis of Cancer.

Early diagnosis implies identification of the presence and location of malignant cells at a time when curative treatment can be performed. Today none of the immunologic tests for cancer-associated antigens are sensitive and specific enough to meet this requirement.

The nature of the immunologic tests for cancer-associated antigens is qualitatively different from tests measuring levels of liver enzymes or bone enzymes. In the latter tests, it is unlikely that a small mass could raise the level of normally present enzymes to an abnormal level; that is, there is a high background of normal activity that obscures the similar activity of the neoplastic cells. In the immunologic tests, the search is for fetal antigens in which the background levels should be low. This is an area in which future progress may produce valuable results.

Immunologic Treatment Monitoring Aids.

Three radioimmunoassay tests are currently of great value in the management of patients with cancer.

The carcinoembryonic antigen (CEA) test is of great assistance in management of some patients with colorectal carcinoma. Where the level is elevated preoperatively, the postoperative levels are useful in as-

sessing the recurrence of disease and the response to therapy. We are not recommending adjuvant postoperative chemotherapy, but an elevation in the CEA is one indication to search for the location of the recurrence and for initiation of therapy.

Radioimmunoassay of chorionic gonadotropins has long been known to be essential in planning the treatment of choriocarcinoma. The radioimmunoassays for alpha-fetoprotein and for the B-subunit of chorionic gonadotropin add a major new dimension to our management of patients with testicular cancer. Decisions about starting chemotherapy and the selection of the chemotherapy drugs used are greatly influenced by the results of these immunologic tests.

Immunotherapy of Cancer.

I have not allotted much space to the analysis of cancer immunotherapy. Many techniques—some simple, some complex, and some very ingenious—are being used to either treat human cancer or to prevent its recurrence.

The experimental studies of BCG immunization in the guinea pig[33] illustrate the potential value and the limitations of immunotherapy. In this system, injection of living *Mycobacterium bovis* BCG into the tumor residing in an animal capable of developing cellular reactivity to BCG, *and* at a time when the tumor is small, results in a marked decrease in the number of tumor-transplantation takes. Many experimental animal systems carefully designed to demonstrate an effect of immunotherapy have been published. The literature on human cancer immunotherapy trials is enormous; its abundance makes it difficult to discount. In my view the effectiveness of any immunotherapeutic procedure in human cancer has yet to be demonstrated. There is great interest in studying the results reported at the conference "Immunotherapy of Cancer: Present Status of Trials in Man."

Predicting the future course of immunotherapists is hazardous. The mood or moving spirit seems to indicate a great disenchantment with BCG and its allied products. A shift to *Corynebacterium parvulum* is underway, but it is probably too toxic to gain wide acceptance. The newest bacterial entry is the pseudomonas vaccine. The direction seems to be to go through Bergey's Manual, a task that could involve generations.

Ironically BCG is being rejected as uncritically as it was accepted. If we are to be able to interpret an immunotherapy trial properly, we need to know more than the change in size of a cancer mass or the duration of survival. We need measurements of the changes in the levels of antitumor blocking antibody, unblocking antibody, and cellular cytotoxicity and cellular suppression. With this information we can learn how to stimulate selectively the portion of the immune response that destroys cancer without stimulating the immune response that aids cancer.

Conclusions.

Immunology provides a valuable tool as a treatment monitoring aid in many cancers.

The likelihood of an effective cancer vaccine is remote and requires identification of both an oncogenic virus and a susceptible subgroup.

The failure to find cancer antigens and the abundance of cancer-associated antigens suggest that cancer may be a programming error and potentially reversible.

Immunology is likely to provide better and effective early diagnostic tests.

The major need in immunotherapy is laboratory support to measure the effects of therapy upon antitumor immune response.

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SCRIPTA MEDICA

Association of Cystic Medial Necrosis of the Aorta and Undiagnosed Thyroiditis

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Introduction.

We have recently seen two patients with cystic medial necrosis of the aorta. The first patient died of a dissecting aneurysm of the thoracic aorta. At autopsy, classical Hashimoto's thyroiditis was discovered. The second patient died of a rupture of the ascending aorta. At autopsy, chronic thyroiditis was seen with multiple large germinal centers and diffuse fibrosis. Neither patient was clinically suspected of thyroid dysfunction although the second patient had had a partial thyroidectomy in the remote past.

The association of dissecting aneurysm and post-thyroidectomy myxedema in three patients was reported many years ago.[1] Autoimmune thyroiditis with symptoms of hypothyroidism and dissecting aneurysm have also been observed together.[2] It has also been reported that aortic aneurysm appears to be significantly more frequent in myxedematous patients than in other hospitalized patients.[3] The underlying theme in these clinicopathological correlations appears to be that hypothyroidism may cause weakening of the aortic wall. This report is meant to focus attention on this possibility and point out that subclinical cases of hypothyroidism may also involve the aorta.

Case Report 1.

A 76-year-old black woman who had a history of mild hypertension was brought to the emergency room because of sudden low back pain and "spells" earlier the same day. Her vital signs were a pulse of 68/min, blood pressure 120/84 mmHg, respiration 44/min. She had prominent epigastric aortic pulsations. A chest x-ray showed cardiomegaly and a widened mediastinum probably due to the thoracic aorta. She had multiple PVB's on her electrocardiogram. She suddenly became unresponsive after her physical exam and died.

The autopsy was performed 17 hours after death. There was a dissecting aneurysm of the thoracic and abdominal aorta and a hemopericardium. The aorta showed mild to moderate atherosclerosis, but the thoracic and abdominal aorta were grossly pliable. Marked cystic medial necrosis was seen with hematoxylin and eosin staining and was confirmed by the significant metachromasia seen with the aldehyde fuchsin and toluidine blue stains. The heart weighed 450 gm and the left ventricle was 2 cm thick. Moderate atherosclerosis of the major coronary arteries was present and the myocardium had moderate diffuse interstitial fibrosis. The thyroid had a diffuse lymphocytic infiltrate together with germinal centers and Hürthle cell change. The morphological criteria for Hashimoto's thyroiditis were therefore fulfilled.

Case Report 2.

A 55-year-old black woman experienced the sudden onset of severe sharp midepigastric pain while ascending a flight of stairs and immediately lost consciousness. She was brought to the emergency room in a state of shock. After admission the patient had generalized seizures followed by episodes of ventricular tachycardia and ventricular fibrillation. She responded to resuscitation at first but later developed cardiac arrest and died. She had no history of hypertension and had been taking no medications. Ten

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years prior to this episode she had undergone a partial thyroidectomy for thyroid nodules.

An autopsy was performed four hours after death. A large rupture in the wall of the ascending aorta extended into the adventitia of the aorta and pulmonary arteries. A hemopericardium containing 100 cc of blood and clot was present. The abdominal and thoracic aorta were markedly atherosclerotic. Marked cystic medial necrosis was seen in the ascending aorta and significant metachromasia was demonstrated with the toluidine blue stain. The heart weighed 450 gm and showed left and right ventricular hypertrophy. The left anterior descending coronary artery was 50% occluded by atherosclerosis within 1 cm of the coronary ostia. Scattered areas of early myocardial necrosis were seen microscopically. The thyroid was small and firm, and one lobe had been previously removed. A marked chronic thyroiditis composed of diffuse lymphocytic infiltration, prominent germinal centers, and scarring was present, but there was no Hürthle cell change. The partial thyroidectomy done ten years previously showed the same histologic pattern. A single sigmoid kidney was present on the right as a result of crossed renal ectopia with fusion.

Discussion

The two patients in this report both had cystic medial necrosis of the aorta and died as a result of complications of the weakened aortic wall. Both patients were hypertensive based either on history or on the autopsy findings of cardiomegaly and left ventricular hypertrophy. Systemic hypertension is often present in patients dying of dissecting aneurysms[4] and ruptures of the aorta. Whether the hypertension has a causative role in cystic medial necrosis or only functions to propagate a tear is not fully understood.

Both of the patients in this report had undiagnosed thyroid diseases at autopsy. In the first patient Hashimoto's thyroiditis involved the entire gland. The second had had a partial thyroidectomy and the remaining thyroid tissue showed chronic thyroiditis. There was diffuse fibrosis and multiple germinal centers, but no Hürthle cell change was seen in this patient and we were therefore reluctant to classify this as a classical Hashimoto's thyroiditis. The anatomic evidence suggests that both of these patients could have had episodes of hypothyroidism which were not detected, so-called preclinical myxedema.

The effect of total thyroidectomy without thyroid hormone replacement in hypertensive patients has been documented by Kountz and Hempelmann.[1] Two patients died of dissecting aneurysm within six months. A third patient was treated with desiccated thyroid for two years. She stopped taking her replacement hormone and ten months later died of dissecting aneurysm. All three patients had cystic medial necrosis. The findings suggest that hypothyroidism is a severe risk factor in the development of dissecting aortic aneurysm in hypertensive patients.

More recently an association of Hashimoto's thyroiditis and dissecting aneurysm was reported.[2] This case involved a patient who was also hypertensive and clinically hypothyroid. Thyroid auto-antibodies were present. Medial degeneration was not documented, but severe atherosclerosis was present. An association of hypothyroidism and abdominal aneurysm has also been shown by Niarchos and Finn.[3] They suggest that preclinical myxedema patients might be picked up with a more sensitive screening test, such as with antithyroid antibodies or serum thyroid stimulating hormone (TSH) levels. In addition, we suggest that patients with aortic aneurysm might be screened more carefully for evidence of preclinical myxedema. In such patients, further weakening of the muscle wall might possibly be prevented.

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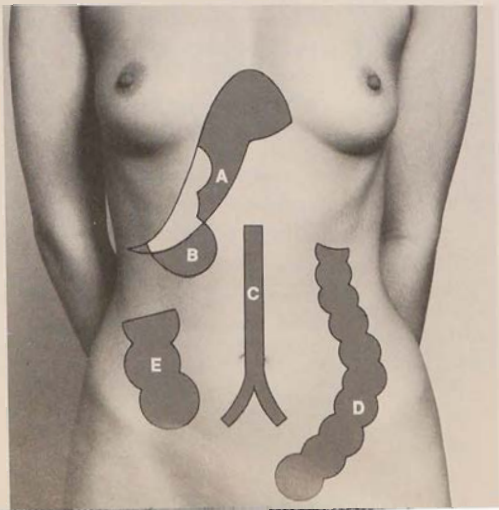
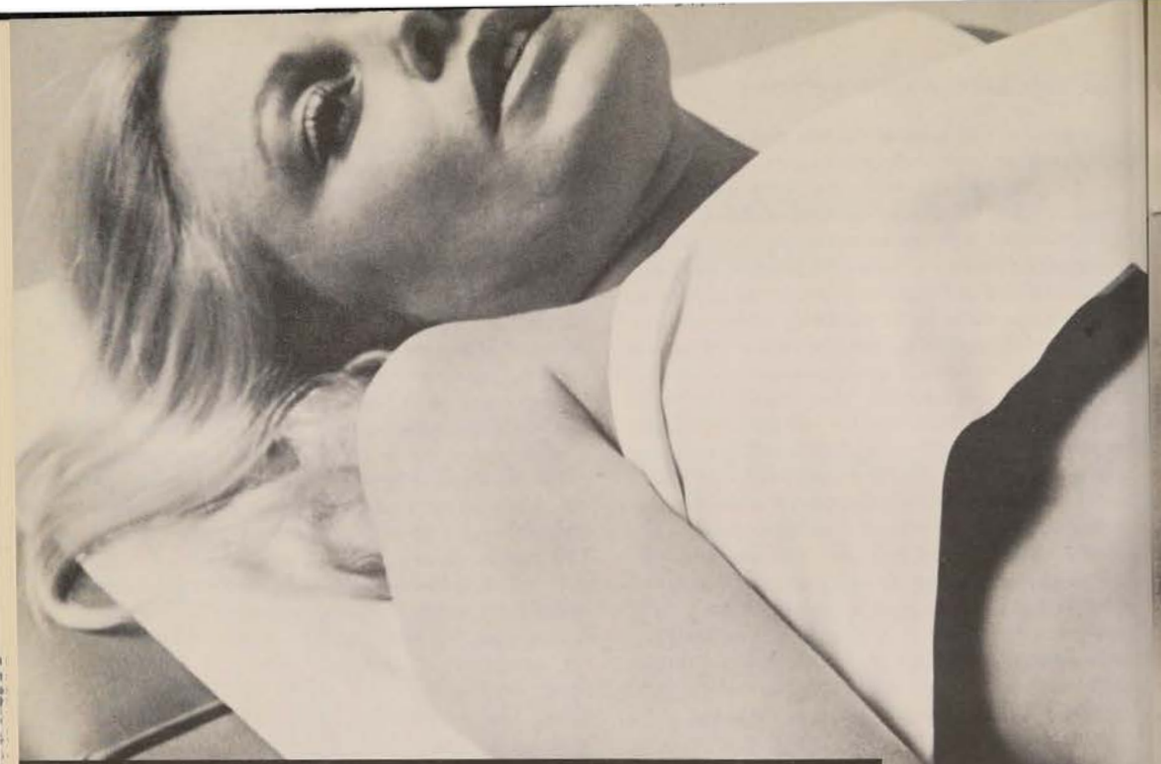
Impossible to outline, unless diseased, distended or enlarged: the gallbladder, pancreas, stomach, small intestine, transverse colon and spleen.





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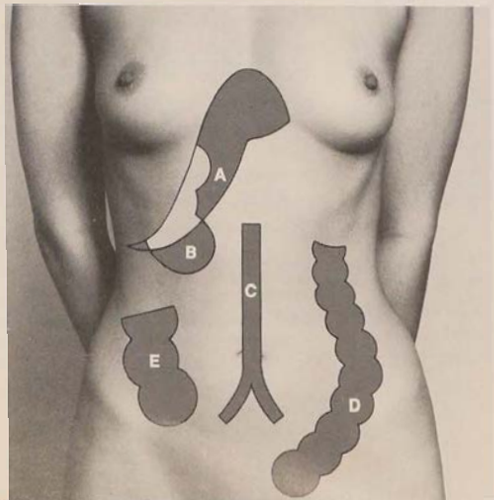

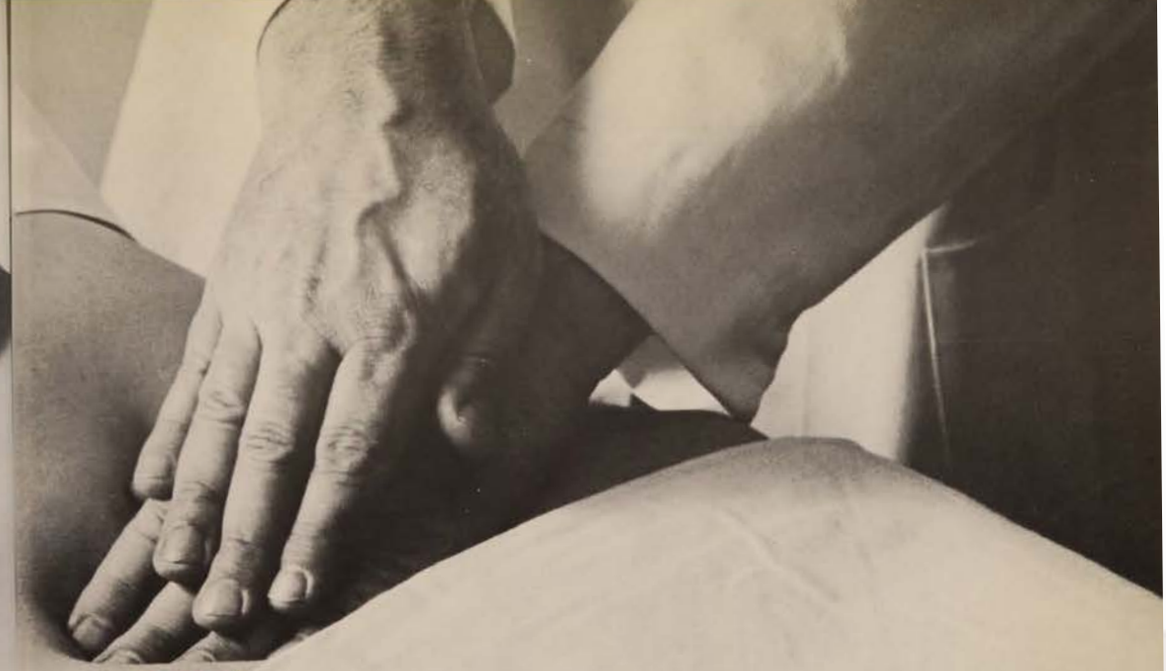

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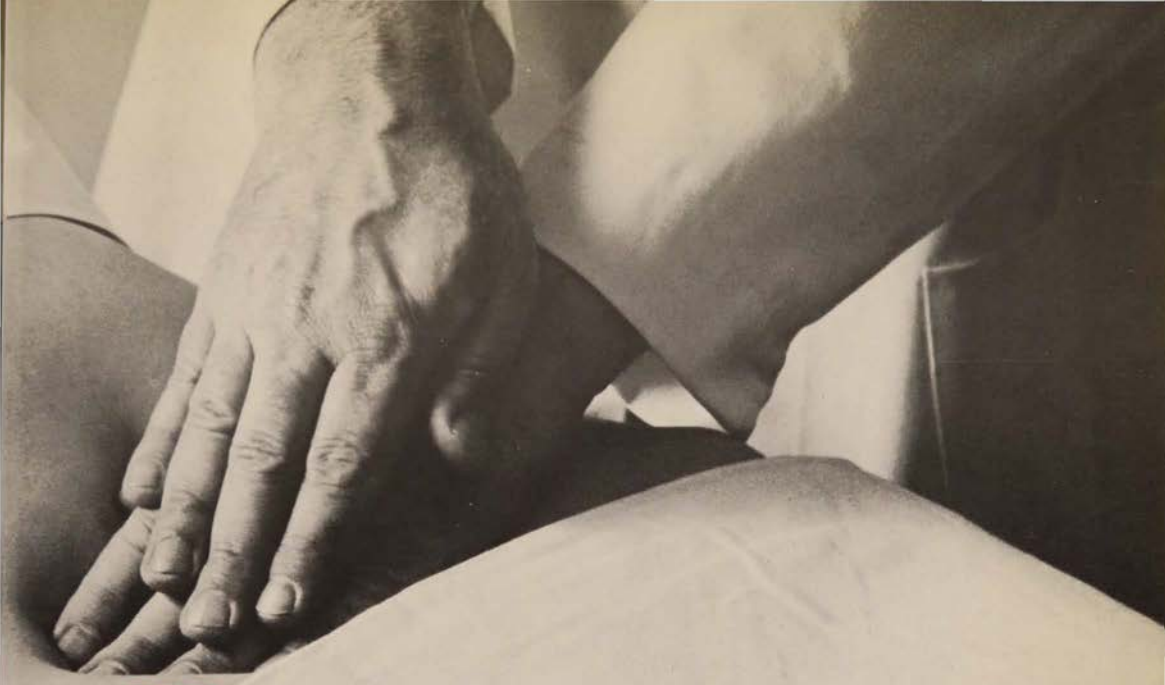

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