The Character and Specificity of an IgA Rheumatoid Factor

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A highly purified IgA rheumatoid factor of restricted heterogeneity has been isolated from a patient with systemic lupus erythematosus and hypergammaglobulinemic purpura. Preliminary studies of this patient's serum revealed serum IgG, IgA and IgM levels of 2900, 985 and 72 mg/100 ml respectively, by radial immunodiffusion; a micro-hemagglutination titer of 1:20,000 against human erythrocytes sensitized with human γG by bisdiazotized benzidine; and the presence of a sharp 9S (uncorrected) spike on Model E analytic ultracentrifugation. Separation of the serum on SG-200 demonstrated that the latex agglutinating activity was confined to the first peak eluted and the adjacent “intermediate” area. With the use of a bromacetyl cellulose–HγG immune adsorbant IgG, IgA and trace amounts of IgM rheumatoid factors were isolated from the serum. The IgG and IgA were separated on DEAE cellulose, and both yielded latex activity. The IgG rheumatoid factor contained both κ and λ light chains and γ1, γ2 and γ4 heavy chains. Sixty-five percent of the protein recovered from the DEAE consisted of IgA. This sedimented as a 7S protein (uncorrected) contained only λ light chains and was resistant to reduction in 0.3M 2ME, alkylation in 0.36M iodoacetamide and dissociation in 1.0M acetic acid. Isoelectric focusing of the IgA rheumatoid factor over a 3–10 pH range showed a sharp and narrow spike with a peak at pH 4.62. Reactivity of the IgA was demonstrated for unaltered Cr (IgG1) and Zu (IgG3) Fc fragments utilizing Sephadex equilibrium molecular sieving. Maximum average intrinsic association constants of both subclasses for the IgA rheumatoid factor were nearly equivalent and of the order of 0.5 × 104 L/M.

These studies demonstrate a rheumatoid factor of the IgA immunoglobulin class which is apparently homogeneous, reacts well with undenatured
heavy chain proteins of two IgG subclasses, and constitutes the major rheumatoid factor component in the serum from which it was derived. This protein should be useful in future studies to determine the precise definition of an autoantigenic site on the IgG molecule.

**Radioisotopic Evaluation of Salivary Gland Dysfunction in Sjögren's Syndrome (SS)**

**DONATO ALARCÓN-SEGOVIA, YOLANDA GONZÁLEZ-JIMÉNEZ, LUIS RENÉ GARZA and JORGE MAISTERRENA, Mexico City, Mexico**

Salivary glands, like the thyroid, can selectively concentrate elements of Periodic Group VII and secrete them in saliva. This capability has been utilized to evaluate salivary gland function in 16 patients with SS and in 7 controls by means of radioisotopic studies with Technetium-99m pertechnetate ($^{99m}$ Tc).

Scintiscans and direct counts over parotid and submaxillary glands done within 30 min of intravenous injection of 1.4 mc of $^{99m}$ Tc showed lower uptake in SS patients (including those without xerostomia) than in controls. Counts were similar over both parotids in all controls, while 11 of the SS patients had significant difference of uptake (>10%) between both parotids.

Except for 2 with low thyroid uptake, SS patients had ratios of thyroid/parotid uptake that were, as an average, twice higher than controls.

Concentration of the isotope in saliva diminished from the 15 to the 60 min postinjection samples in all normal controls, while it increased in all but 3 of the SS patients. These 3 SS patients had lower initial concentration in saliva than the rest; their salivary gland uptake was extremely low; and they all had severe and long standing xerostomia.

Radioisotopic evaluation of salivary function in SS has thus far taught us that (1) there may be salivary dysfunction despite absence of xerostomia; (2) there are two distinct stages of functional disturbance of the salivary glands; and (3) first stage functional salivary disturbance may be reversed, at least partially, with steroid treatment.

**Isoniazid Acetylation Rate and Development of Antinuclear Antibodies (ANA) upon Isoniazid Treatment**

**DONATO ALARCÓN-SEGOVIA and EUGENIA FISHBEIN, Mexico City, Mexico**

Some side effects of isoniazid are dependent on its rate of acetylation which is phenotypically determined and shared by hydralazine. Development of ANA upon isoniazid treatment might be similarly influenced. Of 153 tuberculous patients on isoniazid who were studied, 78 were phenotypically slow and 75 were phenotypically fast isoniazid acetylaters. ANA to nuclei, nucleoprotein (NP), soluble nucleoprotein (sNP), and isoniazid-altered soluble nucleoprotein (sNP/INH) were sought in them by complement fixation tests. Results are shown in the Table.

Although ANA to all antigens were more frequent in slow acetylators, this was not, or was only borderline, statistically significant.

There was no significant difference in incidence of ANA to any of the antigens between patients who had taken isoniazid for over 6 months and those who had lower intake, regardless of their rate of acetylation. ANA developed in fast acetylators on isoniazid for less than 2 months.

These observations support the contention that development of ANA in tuberculous patients on isoniazid is related to alteration of sNP by the drug. They indicate that in vivo, such alteration must occur promptly and rather systematically, thereby resulting in development of ANA to sNP/INH in most individuals. Other ANA may be due to cross reactivity and/or increased immunologic reactivity. Under such circumstances the pharmacogenetics of isoniazid plays little, or no role, in determining the development of ANA upon its intake.
Rubella Vaccination and Acute Arthritis in Women
F. Paul Alepa, Rosvida Sanga and Joseph A. Bellanti, Washington, DC

Twenty-six healthy women, seronegative for rubella antibodies, received 2 different injections of rubella virus vaccine derived from the same strain (HPV-77DK-12 Phillips Roxane, Inc). One vaccine is more attenuated (R-10) and does not lead to measurable antibody production while the other, (R-8), does. Initially, 14 women received one vaccine (R-8) and 12, the other (R-10). After 6 weeks, a crossover was done so that each woman received both vaccines. Rheumatoid factor (RF), antinuclear antibody (ANF) and rubella hemagglutination-inhibition (HI) antibody titers were done prior to vaccination and monthly thereafter. A joint examination was done in each subject and repeated when articular symptoms developed.

Seven of the 26 women (27%) developed arthritis. Four others had vague articular complaints lasting no more than 1 day. Arthritis occurred only after vaccination with R-8 and only in those with a rubella HI titer > 1:16. Of the 14 who received R-8 as the initial injection, 5 (35%) developed arthritis. In contrast, arthritis occurred in only 2 of the 12 who received R-8 as the second injection.

The interval between vaccination and onset of arthritis was 14 to 35 days (mean 23 days). The joints involved were: wrists (5), metacarpophalangeal (5), proximal interphalangeal (5), knees (5), ankles (3). Carpal tunnel (CT) syndrome developed 2 to 3 weeks after the onset of arthritis of the hands in 4 women. Conduction times done in one subject with classical CT symptoms were normal. In 2 women, small knee effusions occurred. Articular symptoms were well controlled by aspirin and resolved without residua in 10 to 35 days. Two women developed ANF during the study. Neither had arthritis. Only one subject had a significant titer rise in RF (1:16 to 1:128). She also developed arthritis with CT syndrome which resolved within 30 days. The RF titer, however, remained elevated.

The incidence of arthritis following rubella virus vaccination in adult women is significantly greater than that reported in children (0.42%). Although it was severe in most of the women it was self limited and well controlled by aspirin.

Local Production of "Auto Antibodies" in Recurrent Parotitis of Childhood
F. Paul Alepa and Joseph A. Bellanti, Washington, DC

Intermittent recurrent parotitis (IRP) of childhood is a local inflammatory disease of unknown etiology. The parotid saliva from patients with this disorder offers an opportunity to study the secretions from an area of local inflammation. Rheumatoid factor (RF) and fluorescent antinuclear antibody (ANF) were measured in the parotid saliva and serum from such patients.

| Patients | Parotid saliva | Serum |
|---------|----------------|-------|
|         | Right | Left |     |     |     |     |
| JO      | 0     | 0    | >1024* | +    | 256 | 0    |
| OG      | 80*   | 0    | 40    | 0    | 0   | 0    |
| SK      | ND    | ND   | >1024*| +    | 0   | +    |
| FW      | 160*  | +    | ND    | ND   | 0   | 0    |
| SV      | >1024*| +    | >1024 | +    | 0   | 0    |
| SD      | >1024*| 0    | 512*  | 0    | 0   | 0    |

ND = Not done.
* Affected parotid gland.

Six children with IRP of at least 1 year's duration were studied. None had evidence of articular or ocular disease. Five had only unilateral and 1 had bilateral involvement. Three had acute involvement at the time of the study. Bacterial and viral agents were not isolated.

RF was detected in the parotid saliva of all 6, but in the serum of only 1 (JO) and then in lower titer. Similarly 4 had ANF in the saliva and only 1 in serum (SK). The suggestion of local production of these antibodies is further supported by the failure to detect either of them in parotid saliva from the unaffected gland in JO. Fractionation of JO's saliva by sucrose gradient revealed RF and ANF in both heavy and light fractions. In contrast, serum RF was detected only in the heavy fraction. In SV and OG, who had unilateral disease, one or both of these antibodies were found in the saliva from the unaffected gland. The mere presence of RF and ANF does not appear to be sufficient to cause symptomatic parotitis. RF and ANF were not detected in the parotid saliva or serum of 2 patients with classical mumps.

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**Arthritis Associated with Hepatitis: Complement Component Studies**

ELLIOT ALPERT, RUTH L. COSTON and PETER H. SCHUR, Boston, Massachusetts

The symptom complex of arthritis, fever and/or rash, frequently associated with viral hepatitis, may be a serum sickness syndrome due to circulating immune complexes. Total serum complement CH$_{10}$ and 3 of the early complement components (C'1, C'4, C'3) were measured in the serum of 50 patients with acute inflammatory diseases of the liver. Total serum complement activity was measured by the hemolytic method, and the 3 components were measured by quantitative radial immunodiffusion using monospecific antisera. The CH$_{10}$ and the 3 components measured tended to be normal or high in a variety of acute hepatic diseases including those drug-induced and some cases of acute and chronic viral hepatitis. However, the CH$_{10}$ and 1 or more of the components studied were significantly depressed in 7 patients with acute viral hepatitis. All of these patients had arthralgias or arthritis involving the distal joints and usually the PIP joints with pain, morning stiffness, and occasionally erythema and small effusions. This was associated with either fever or urticarial rash. This syndrome occurred in the prodrome or early stages of the hepatitis. All 7 patients had circulating hepatitis-associated (HAA, SH, Au) antigen demonstrable in the serum. Activation of the complement system by circulating immune complexes, presumably the hepatitis virus and homologous antibody, may be involved in the pathogenesis of the arthralgias or arthritis associated with viral hepatitis.

**Evaluation of Xerostomia by Salivary Gland Scintigraphy in Patients with Sjögren's Syndrome (SS)**

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The diagnosis of xerostomia in SS depends largely upon clinical appraisal, with supplementary data from salivary flow rates (SFR), sialography and salivary gland biopsy. An objective estimation of salivary glandular function is provided by scintigraphy with Technetium$^{99m}$, which, as pertechnetate ion, is selectively taken up and concentrated by salivary glands and secreted into the saliva.

Twenty patients with SS were injected intravenously with 10 mCi of Technetium$^{99m}$-pertechnetate. Sequential 2-min scintiphotos were obtained for the first 12 min and then every 10 min for the next 60–80 min. Data were recorded on video tape for later quantification. Comparable control studies were done during routine technetium brain scans on patients with no known salivary gland disease.

The pattern of pertechnetate uptake, concentration and excretion by major salivary glands correlated with clinical grading of disease severity and with SFR. Patients with severe xerostomia and absent or low SFR displayed absent or delayed uptake of radioisotope, markedly diminished concentration, and delayed excretion into the oral cavity. Patients with minimal symptoms and near normal SFR had relatively normal salivary dynamics but decreased absolute levels of isotope concentration. Unilateral predominance of parotid dysfunction in some patients was detected by both SFR and scintigraphy. When there was sufficient uptake of pertechnetate to permit visualization, four-view scintiphotos provided estimation of parotid and submandibular gland structure and size. Sialography, lower lip biopsy histopathology, and antisalivary duct antibody correlated less well with clinical severity.

Salivary gland scintigraphy is an easy, safe, objective, and accurate method for evaluating xerostomia and may prove useful not only in diagnosis but also in monitoring disease progression and response to therapy in SS.

**Radiological Changes in the Hip Joint After 10 Years of Juvenile Chronic Polyarthritis**

BARBARA M. ANSELL and METIN UNLU, Taplow, England

At the 10 year follow-up of 235 cases of juvenile chronic polyarthritis, all except 9 had a pelvic radiograph performed. These films, together with 50 juvenile pelvic films obtained from the Azoos population survey were read onto a specially designed form.
Forty percent of the 93 male pelvic films and 48% of the 133 female films from the patients had one or more abnormalities. These were unilateral in 7 males and 13 females. Growth anomalies involving both the femoral head and acetabulum were very frequent in those whose disease commenced under the age of 5, and whose disease process had remained active for more than 5 years. These features became less marked as the age of onset advanced. Underdevelopment of the femoral head was the most common, but overdevelopment did occur, and both were associated with flattening of the head. Migration upwards of the femoral head together with straightening of the femoral neck was more common in the younger patients; protrusio was uncommon. Bony fusion of the joint had occurred in 4 patients, being bilateral in 1.

As these observations were made on cases which form part of a long-term prognostic study, the natural history of the changes can be demonstrated.

**Interaction of Rheumatoid Factor with Infectious Herpes Simplex Virus–Antibody Complexes**

**Warren K. Ashe, Charles A. Daniels, G. Stuart Scott and Abner L. Notkins, Bethesda, Maryland**

Earlier studies showed that infectious virus-antibody (VA) complexes could be neutralized by specific anti-immunoglobulins. Since rheumatoid factor (RF) is an IgM immunoglobulin which reacts with IgG immunoglobulins, the present investigation was undertaken to see whether infectious herpes simplex virus (HSV)–antibody complexes could be neutralized by RF.

RF was prepared from the precipitated euglobulin fraction of human rheumatoid sera (latex positive) by chromatography at pH 4.2 on Sephadex G-200. The IgM peak was RF-positive but had no anti-HSV activity, while the IgG peak was RF-negative and had anti-HSV activity. Other human and rabbit sera containing antibody to HSV were chromatographed on Sephadex G-200 and the IgG fractions were used as the source of anti-HSV antibody. Human or guinea pig sera with no detectable anti-HSV antibody and RF-negative by latex agglutination served as sources of complement (C').

Infectious VA complexes were prepared by incubating HSV with appropriate concentrations of anti-HSV IgG and infectivity was determined by the plaque technic on primary rabbit kidney cell monolayers. Incubation of these infectious VA complexes with RF alone or with C' alone failed to produce neutralization. Evidence that RF had attached to the infectious VA complexes came from experiments which showed that antibody made in goats against human IgM neutralized VA complexes that had been incubated with RF, but it did not neutralize VA complexes that had been incubated with IgM from nonrheumatoid sera. Although RF alone failed to produce neutralization, the addition of C' to reaction mixtures containing VA complexes and RF resulted in substantial neutralization (> 90%). The importance in this system of the Fc portion of anti-HSV IgG was illustrated by the fact that VA complexes prepared with the Fab I fragment of rabbit anti-HSV IgG were not neutralized by RF and C'.

The relationship between RF and the fixation of C' and the mechanism of neutralization of infectious VA complexes by RF and C' remain to be determined. The demonstration that RF can interact with infectious VA complexes points to the possibility that VA-RF complexes might be involved in immune complex disease and possibly in the pathogenesis of rheumatoid arthritis.

**β-Mannosidase Activity in Synovial Fluid**

**B. Bartholomew and A. Perry, Denver, Colorado**

While a number of acid hydrolytic glycosidases have been studied in synovial fluid and tissue, no previous evaluation of the properties of β-mannosidase activity in human inflammatory disease has been carried out. The purpose of the present study is threefold: (1) the delineation of the kinetic properties of this enzyme in synovial fluid and the development of a microassay system; (2) the establishment of β-mannosidase activity as distinct from other synovial fluid glycosidases; and (3) the measurement of β-mannosidase activity in the synovial fluid of various arthropathic conditions. The synthetic glycoside, p-nitrophenyl-β-mannoside, was used in all the present studies. In synovial fluid, with this substrate, the β-mannosidase activity had a $K_m$ value of $3.4 \times 10^{-4}M$ and could be assayed in...
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5-25 μl of synovial fluid with a linear response for at least 90 min in an acetate buffer system, pH 3.5. Substrate competition experiments at saturation showed 90% or better additive results with the appropriate synthetic glycosides for β-N-acetylglucosaminidase, β-glucuronidase and α-mannosidase. Heat stability studies resulted in abolishment of β-mannosidase activity with preservation of 60% of the α-mannosidase activity. Twenty-six nonbloody, synovial fluid samples showed the following levels of activity in μM of p-nitrophenol released/milliliter of synovial fluid/hour of incubation: rheumatoid arthritis (12 samples) 0.67, gout (5) 0.76, pseudogout (4) 0.35 and traumatic or osteoarthritis (5) 0.20. These studies suggest that β-mannosidase activity is a distinct entity in synovial fluid and that levels of activity correlate with the degree of inflammation present.

Recurrent Synovitis Following Synovectomy of the Knee: A Clinical, Histologic and Biochemical Correlation

B. BARTHOLOMEW, D. MILLS and M. PATZAKIS, Denver, Colorado

Synovectomy of the knee gives subjective and objective improvement in nearly all patients with rheumatoid arthritis. The number of clinical recurrences increase with duration of follow-up. In the present study, histologic and biochemical evidence was obtained to document these impressions. Synovial fluid and tissue specimens were obtained in 32 patients, 2 to 120 months after synovectomy.

The clinical evaluation consisted of a search for joint effusion and acute inflammation and x-ray comparison of pre- and post-synovectomy films. The histologic evaluation was done on specimens prepared by routine methods for electron microscopy. The simultaneous presence of lining cell hypertrophy, lining cell hyperplasia and perivascular plasma cell infiltration was called a recurrence. These criteria best separated rheumatoid controls, who were unoperated, from osteoarthritic, traumatic and amputation controls. Biochemically, synovial fluids were evaluated for β-N-acetylglucosaminidase, β-glucuronidase, β-galactosidase, and α-mannosidase activity. These results were compared with those from traumatic and osteoarthritic effusions for each enzyme. A value one-third higher than the average obtained in the controls constituted evidence of active inflammation.

| Months since synovectomy | Results | (No. of patients) |
|--------------------------|---------|------------------|
| 0-11                     |         | (11)             |
| 12-35                    |         | (9)              |
| 36-120                   |         | (12)             |
| Clinical recurrence      | 9%      | 40%              |
| Histologic recurrence    | 36%     | 78%              |
| Biochemical recurrence   | 50%     | 75%              |

These results suggest that measurable synovial membrane and fluid changes occur prior to the development of subjective or clinical recurrence of active synovitis and may be early indicators of recurrent disease. These data indicate that synovectomy in many rheumatoid arthritis patients provides only temporary benefit.

The Rarity of Ankylosing Spondylitis in the Black Race

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The influence of hereditary factors for ankylosing spondylitis (AS) has been fairly well established by a number of studies for the white race. There are, however, no studies of the distribution of AS in the black race. We have collected from 4 VA hospitals in different areas of the country the frequency rates for the diagnosis of AS in black and white males. These have been compared to hospital admission rates and population distribution in the environs of the hospitals. At the Dallas VA Hospital, where comparisons were made with rheumatoid arthritis (RA) and Reiter’s syndrome, the white:black admission ratio was 4:2. The ratio for RA was 5:5; for Reiter’s syndrome, 1:8; and for AS, 13:7. In the other hospitals data were only obtained for AS. In Richmond, Va, the white:black admission ratio was 1:8 and the ratio for AS 20:0. In Pittsburgh, Pa, the white:black admission ratio was 5:7 and the ratio for AS 14:7. At the New York VA Hospital, the admission rate was 3:0 and the rate for AS 6:5.

The combined data for the 4 geographically separated VA hospitals shows the white:black ratio of AS to be 13:7. This compared to the hospital admission ratio of 3:2 shows that AS appears in blacks with only 25% of the frequency found in whites. A
close correlation with this figure is found in blood typing studies for the percentage of white genes in the black Afro-American, which is also about 25%.

A review of the African literature supports these data by confirming the apparent rarity of AS in the black African. Thus the appearance of AS in the black Afro-American is apparently related to his complement of white genes. These findings support a genetic factor in the etiology of ankylosing spondylitis.

Alteration of Pyrimidine Metabolism Resulting from Allopurinol Therapy

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The effect of allopurinol on the urinary excretion of ultraviolet absorbing compounds was assessed in 6 gouty patients by the use of an automated high pressure anion exchange column coupled with an ultraviolet detection system. In all patients studied, allopurinol administration led to the expected findings which included (1) a decrease in uric acid excretion, (2) an increase in the excretion of hypoxanthine and xanthine and (3) the excretion of allopurinol as well as its known metabolites, oxipurinol and allopurinol ribonucleoside. An unexpected finding was a marked increase in the excretion of the pyrimidines, orotidine and uridine acid. Urinary orotidine increased from a mean of 7.9 mg/day to a mean of 49.7 mg/day. Urinary uridine acid increased from < 2.0 mg/day to a mean of 16.8 mg/day. These findings suggested that allopurinol was interfering with the further metabolism of orotidylic acid (OMP).

Orotidylic decarboxylase which catalyzes the conversion of OMP to uridylic acid (UMP) was assayed in dialyzed human erythrocyte lysates by following the liberation of CO₂ from carboxyl labeled ¹³C-OMP. Allopurinol ribonucleotide (Ki = 1 × 10⁻⁹M) and xanthyllic acid (XMP, Ki = 1 × 10⁻⁹M) were potent inhibitors of orotidylic decarboxylase in a manner competitive with respect to OMP (Km = 1.5 × 10⁻⁹M). Xanthine, hypoxanthine, allopurinol, and oxipurinol had no inhibitory effect on this enzyme.

We conclude that the administration of allopurinol, in addition to inhibiting uric acid synthesis, appears to substantially alter pyrimidine metabolism. The finding that allopurinol ribonucleotide and xanthyllic acid are potent inhibitors of human erythrocyte orotidylic decarboxylase, an enzyme essential for de novo pyrimidine biosynthesis, provides a mechanism to account for this effect.

Massive Osteolysis of the Femoral Head in Rheumatoid Arthritis, Treatment by Total Hip Replacement

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The disappearance of skeletal bone has been reported in many entities. Although dissolution of bone in rheumatoid arthritis (RA) is well known, rapid and massive osteolysis is an unusual finding. This is a report of a patient with long-standing RA in whom not only was there low grade bone resorption at multiple joint sites, but who in addition developed rapid, massive osteolysis of the head and neck of the femur over a 3-month period. The patient subsequently underwent total hip replacement with a Charnley type low friction prosthesis cementing the femoral and acetabular components to the osteoporotic bone with methyl methacrylate. It is felt that this patient does not fall into the usual categories associated with massive osteolysis. It is particularly interesting that various joints showed a range of severity in the degree of bone destruction climaxing by the total disappearance of the femoral head under surgical and radiological observation. It is speculated that this patient exhibited the often described bone resorption of RA in its most extreme form.

Serum Anti-γ Globulins in Juvenile Rheumatoid Arthritis (JRA)

NICOLAS E. BIANCO, RICHARD S. PANUSH, J. SYDNEY STILLMAN and PETER H. SCHUR, Boston, Massachusetts

The low incidence in JRA of positive latex fixation tests (LFT), presumably due to IgM anti-γ globulins (anti-IgG), sharply differentiates it from adult rheumatoid arthritis. In this study, sera
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from normal individuals and from JRA's were examined for IgG, IgA and IgM anti-IgG, and by the LFT at 37° C for 1½ hr and at 4° C for 18 hr. Human IgG was insolubilized with glutaraldehyde and incubated with serum; eluates were obtained with pH 2.8 glycine buffer, neutralized, and then assayed for IgG, IgA and IgM content by radial immunodiffusion.

IgG and IgA anti-IgG were detected in both normal and JRA sera; levels were higher in JRA's. IgM anti-IgG were elevated in LFT positive JRA patients. Positive LFT's at 4° C were found in normals and in JRA's whose tests at 37° C were negative; titers were distinctly higher in JRA's.

JRA's with IgM or IgG, although not IgA, anti-IgG levels above 2 standard deviations of the normal group mean, had more extensive disease, including articular inflammation, greater functional disability, and a less favorable course than did JRA's with levels in the normal group range. Similar, though less striking differences were noted for JRA's whose LFT were negative at 37° C and positive at 4° C as compared to patients with negative tests at both temperatures.

| Classification          | No. of patients | Anti-IgG (mean value µgm/ml) | Latex test (%) positive |
|-------------------------|-----------------|------------------------------|-------------------------|
|                         |                 | IgG  | IgA  | IgM  | 4° C | 37° C |
| Normals                 | 50              | 98   | 52   | <20  | 32   | 0     |
| JRA                     | 92              | 147  | 74   | 36   | 62   | 13    |
| latex positive (37° C)  | 15              | 162  | 95   | 100  | 100  | 100   |
| latex negative (37° C)  | 77              | 140  | 70   | <20  | 46   | 0     |

Catabolism of Proteinpolysaccharides by Human Skin Culture Fibroblasts Extracts In Vitro

THEODORE F. BIESIADECKI, MILDRED KISTENMACHER, ROBERT D. CAMPO, ANGELO M. DEGEORGE and VICTOR H. AUERBACH, Philadelphia, Pennsylvania

A number of human diseases of genetic origin are characterized biochemically by the presence of abnormal amounts of one or more mucopolysaccharides (MPS) in urine. It has recently been shown that in some of the mucopolysaccharidoses, the nature of the enzymatic defect prevents normal degradation of MPS. In tissues MPS occur in combination with protein and are known as protein-polysaccharide complex (PPC). Such PPCs have been particularly well studied in rabbit and human leukocytes. Various enzymes derived from bovine cartilage and rabbit hepatic lysosomes have been shown to degrade PPC by attacking either the protein or polysaccharide end of the molecule. The purpose of this communication is to demonstrate the ability of human skin culture fibroblast extracts to catabolize PPC.

Monolayer subcultured skin fibroblasts from 2-oz culture bottles were used. Lysis of cells was accomplished by rapid freezing and thawing. The tubes were then centrifuged and the clear supernatant fluid used as the cell extract. Crude bovine PPC dissolved in phosphate buffers at pH 3.8 or 7.3 was used as substrate. The incubation system used was a diffusion vessel described by Gerber and Schubert (Biopolymers 2:259, 1964). The chambers were separated by a 0.22 mµ Millipore filter. Uronic acid was determined by the Dische method. In addition, 25 µg samples from the output chamber were spotted on paper and separated by high voltage electrophoresis. Controls were also carried out using boiled extract or with no extract. At the end of 4 hr incubation, the amount of uronic acid recovered averaged 120 µg as compared to 10 µg per chamber for the controls. Free chondroitin sulfate was also evident on electrophoresis after incubation in the experimental chambers and not the control chambers.

It is evident from these results that normal human skin fibroblasts grown in tissue culture contain enzymes capable of catabolizing PPC and that these enzymes are not species-specific. It is hoped that these preliminary results will lay the basis for a study of the relationship of these PPC degrading enzymes to the mucopolysaccharidoses.
Use of Selected Clinical and Hematologic Criteria for the Diagnosis of Systemic Lupus Erythematosus (SLE)

THOMAS BITTER, GEORGE J. FRIOU AND EDMUND L. DUBOIS, LOS ANGELES, CALIFORNIA

In an attempt to establish criteria for the diagnosis of SLE, the following 10 features (to be defined in detail) have been examined prospectively in 240 patients with arthralgias, polyarthritis, fever of unknown origin with or without serologic data suggestive of SLE: (1) organic brain syndrome; (2) episodes of reversible loss of hair; (3) a nonscarring facial rash with “butterfly” distribution and/or sun sensitivity; (4) Raynaud’s phenomenon; (5) pericarditis; (6) repeated pneumonias at times of disease activity; (7) repeated pleuritis in the absence of both (5) and (6); (8) repeated urinary findings suggestive of glomerulonephritis; (9) leukopenia and/or lymphopenia and/or thrombocytopenia; (10) coombs-positive hemolytic anemia.

The patients fell into two major groups: (1) Four or more features were found in 117 patients (48.5%) in the absence of any signs suggesting connective tissue disease other than SLE. Two additional patients with proliferative-membranous glomerulonephritis also had erosive polyarthritis, nodules and a high titer of rheumatoid factor. (2) Two or less features were noted in 109 patients (45.5%), all but 5 of whom had obvious signs of other connective tissue disease.

There was an overlap between the two groups consisting of only 12 patients (5%) with three of the above features. A serologic confirmation of a diagnosis of SLE was obtained in 10 of these; none had joint erosions. Two seronegative patients had signs of progressive systemic sclerosis.

Thus, 129 patients had clinical and hematologic features supporting a diagnosis of “definite” SLE. Serologic confirmation was helpful in 10 of these. Only 2 patients with SLE had erosive polyarthritis, the presence of which should probably cast doubt upon a diagnosis of SLE.

Antinuclear Antibody Specificity in Procainamide-Induced Lupus

STEPHEN E. BLOMGREN AND JOHN H. VAUGHAN, ROCHESTER, NEW YORK

This study was designed to determine the specificity of antinuclear antibodies (ANA) occurring in procainamide-induced lupus. The method used was globulin precipitation by salt in the presence of radiolabeled antigen. The antigens used were native DNA, sonicated heat denatured DNA, and sonicated nucleohistone, each labeled with H-actinomycin D. Results are expressed as percentage precipitation of 10 μg antigen added to 0.1 ml serum.

The mean percent precipitated with native DNA was 12% by 27 normal sera, 13% by 35 PSLE sera, and 26% by 27 idiopathic lupus (SLE) sera.

The mean percent precipitated with sonicated denatured DNA was 11% by 19 normal sera, 22% by 32 PSLE sera, and 23% by 12 SLE sera.

Modification of Chemotaxis by Synovial Fluid Hyaluronate

KENNETH BRANDT, BOSTON, MASSACHUSETTS

The chemotactic response of leukocytes in the synovial fluid (SF) presumably plays a significant role in many cases of joint inflammation. While it is clear that the viscosity of fluid from inflamed joints is usually lower than normal and the uronic acid concentration diminished, it is not known whether changes in the character of the synovial fluid affect the movement of WBC’s through the joint space. The present study examines the manner in which synovial fluid hyaluronate (HA) molecules influence the chemotactic migration of WBC’s in their domain.

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HA was isolated and purified from joint fluids of patients with DJD and inflammatory synovitides by precipitation with cetylpyridinium chloride after digestion with Pronase. Samples of purified HA, as well as untreated SF, were chromatographed on Sepharose 2B to provide an index of the size range of the hyaluronate molecules. Chemotaxis was studied in Millipore chambers by a modification of the Boyden technic with normal peripheral blood WBC's in the cell compartments, suspended in buffer or in solutions of purified HA or diluted SF. Test compartments contained the same solutions as the cell compartments, with lyophilized E coli as the chemotactic agent.

The movement of WBC's stimulated by E coli through both SF and solutions of purified HA was markedly diminished, in comparison with their migration through buffer. Fluids giving a "poor" mucin clot with acetic acid impaired chemotaxis less than fluids giving a "good" clot, and HAS isolated from "good" and "poor" fluids varied in the same fashion. Samples of purified HA, and the intact fluids from which they were isolated, when equimolar with respect to uronic acid, inhibited chemotaxis to the same degree. All SFs and solutions of HA were heterogeneous in size on gel chromatography, but samples comprised of greater proportions of larger sized hyaluronate molecules were more viscous and impeded chemotaxis to a greater extent than samples containing lesser amounts of the larger molecules. HA from umbilical cord (a smaller molecule than HA from SF), even at 20 mg/ml, permitted chemotaxis almost as readily as did buffer alone; whereas HA from SF markedly impeded migration at 0.1 mg/ml. The inhibitory effects on chemotaxis of SF and purified HA were both abolished by hyaluronidase.

The data indicate that the physical state of the HA in synovial fluid may significantly modify the response of WBCs to a chemotactic stimulus within the joint space.

**F(ab')2-like Immunoglobulin Fragments in Urines of Patients with Systemic Lupus Erythematosus (SLE)**

**SAMUEL BRODER, JAMES T. CASSIDY, CHARLES B. GOLDBERG, ANN BURT and FRANK WHITEHOUSE, JR, Ann Arbor, Michigan**

The immunoglobulin components present in urine are principally IgG, IgA, light chains and Fc fragments. Urinary F(ab')2-like fragments have not been described by other investigators. In the present study, these fragments were found and characterized in the urines of 5 patients with SLE who were critically ill.

Fresh urine was lyophilized immediately, or was concentrated by precipitation of protein with ammonium sulfate. On immunoelectrophoresis, an extra precipitin arc was detected in the far cathodal region. This arc crossed or was parallel to the IgG arc in 3 cases. It developed with antisera to whole human serum, IgG, F(ab')2, and light chains, but was not detected with antiserum to Fc fragment. It fused in identity around a shortened trough with the arc produced by F(ab')2 prepared by pepsin digestion of IgG. Furthermore, it fused with the cathodal arc of a component in sera and urines of extensively burned patients which has been characterized as an F(ab')2-like fragment. In sucrose density gradient ultracentrifugation, its sedimentation was comparable to a control F(ab')2 fragment. It was not found in the urines of 11 patients less severely ill with SLE or 17 patients with proteinuria of diverse etiology, and was not detected in sera from 100 patients with SLE. The fragment was not produced by addition of urine to serum; however, a similar fragment can result from in vitro degradation of serum that has been repeatedly frozen and thawed.

The urinary fragment described in this study might reflect a poorly understood mode of immunoglobulin metabolism and, as an antigen, could stimulate production of pepsin-site agglutinators. In addition, F(ab')2 fragments do not fix complement and are poor opsonins. F(ab')2-like fragments may, therefore, play an unrecognized role in the pathogenesis of the immune complex diseases such as SLE.

**Relationship Between Mycoplasma Antibodies and Rheumatoid Factors (RF)**

**THOMAS McP. BROWN, HAROLD W. CLARK and JACK S. BAILEY, Washington, DC**

Different types of RF have been characterized as anti-antibodies, yet the identity of the inciting antibodies has been limited. Uncertainty exists concerning the relationship between viral antibody
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Responses and the RF expression. Reports have been given on the high incidence of mycoplasma complement fixation (MC'F) antibodies in the general arthritic population and also on the RF reaction with mycoplasma-antibody complex. In a recent comprehensive 3-year study of 120 arthritic patients, a statistically significant inverse correlation was found between RF and mycoplasma. A high incidence of RF and low MC'F antibody was found in the RA subjects, while a low incidence of RF and a high incidence of MC'F was found in the nonrheumatoid patients. These studies also revealed that 2 of the 7 types of mycoplasma tested appeared related to RF. Some sera also showed a reversal in RF and MC'F antibody over an extended period of observation.

The search for a true animal model of human rheumatoid arthritis has resulted in finding a gorilla with spontaneous rheumatoid-type arthritis. The close serological relationship between this animal model and man may be responsible for producing a statistically significant inverse correlation between MC'F antibody and the RF. The RF level decreased when MC'F antibody production was initiated therapeutically. Further stimulation of MC'F antibody initiated RF expression in sera within a 2 to 3-week period at which time the MC'F became negative. These results suggest that the RF could be spontaneously induced by persistent and appropriate levels of mycoplasma antigen-antibody complex and can also neutralize or be neutralized by adequate levels of MC'F antibody.

Variations in the antigenic properties of mycoplasma grown in different animal tissue broths seem to support the species specific character of RF. Human mycoplasma antibodies also react specifically with mycoplasma grown in human tissues. It is postulated from these results that the persistent and spontaneously induced mycoplasma antibody is a potential autoantigen for a RF found in rheumatoid arthritis.

Immunological Relationship Between "Mycoplasma arthritidis" and Rat Tissues

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Previous studies on rat polyarthritis (Cole et al: J Bacteriol 98:930, 1969) indicated that M arthritidis failed to induce metabolic inhibiting (MI) antibody in rats. M arthritidis was capable of stimulating MI antibodies in other laboratory animals, and nonmurine mycoplasmas were capable of stimulating MI antibodies in rats. A hypothesis is based upon the possible occurrence of a hetero- genetic antigen(s) common to M arthritidis cell membranes and rat tissue was proposed to explain these observations.

Complement fixation (CF), immunofluorescence and agar gel double diffusion tests were used to investigate the proposed immunologic relationship between rat tissues and M arthritidis. Rabbit antisera against 6 strains of M arthritidis exhibited positive reactions in the CF test with an alcohol-saline extract of rat muscle, whereas only 6 out of 18 antisera against other mycoplasma species were positive. Using gel diffusion technics, rabbit and anti-M arthritidis serum failed to react with rat muscle antigen and vice versa. However, absorption of various M arthritidis antigens with antiserum against rat muscle removed at least one precipitin band when the absorbed mycoplasma antigens were reacted against homologous antisera. When antigens of other mycoplasma species were absorbed with antisera to rat muscle, no reduction in the number of precipitin bands was observed. Rabbit antiserum against M arthritidis was conjugated with fluorescein isothiocyanate and reacted against frozen sections of muscle tissues of various animals. As controls, unlabelled normal rabbit serum and rabbit anti-M arthritidis serum were included to determine the specificity of the reaction. Rat, hamster and mouse skeletal muscle exhibited specific fluorescence, whereas chicken, bovine, frog and turtle muscles exhibited no specific fluorescence.

These results indicate the occurrence of a hetero- genetic antigen(s) between M arthritidis and rat tissue. The specificity of this reaction and its possible role in the pathogenesis of rat arthritis will be discussed.

Prognosis in Juvenile Rheumatoid Arthritis (JRA): A Ten-Year Follow-up

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As part of our study of the long-term prognosis of JRA, and following our preliminary 5-year sur- vey (Arthritis Rheum 8:494, 1965), we are reporting the status of 100 patients (62 girls, 38 boys) all of
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whom have now been followed for at least 10 years. The mean age of the 100 patients is 16 years, the range 10 to 28 years. Currently, 40 patients have active disease, 31 of whom have had a course of polyarthritis (Table). Only 1 of 10 patients with a polycyclic acute febrile (benign systemic) course continues to be active, and only 8 of 24 with oligoarthritis (1 to 3 joints), affirming the benign nature of these two patterns of disease course.

Only 11 patients are in the unfavorable ARA functional classes III and IV (Table); each has had a course of unremitting polyarthritis and progressive hip involvement. Two of these patients died, 1 from staphylococcal bacteremia following knee synovectomy, the other from amyloidosis. Statural growth is retarded in 7 patients, 6 of whom also have micrognathia.

Our most striking observation is that chronic iridocyclitis appeared and recurred primarily in patients with the least amount of joint involvement (Table). Of the 8 patients with iridocyclitis, 2 lost vision in one eye: one while in remission from oligoarthritis, the other during active polyarthritis. Although the functional status of these patients is good, recurring iridocyclitis (3 patients), even during remission of the arthritis, carries with it the threat of potential blindness.

| Mode of onset    | No. | Course of disease     | No. | Active disease | Classes III & IV | Chronic iridocyclitis |
|------------------|-----|-----------------------|-----|----------------|------------------|-----------------------|
| Acute febrile    | 20  | Polycyclic acute      | 10  | 1              | 0                | 0                     |
| Polyarticular    | 48  | Polyarthritis         | 66  | 31             | 11               | 2                     |
| Monarticular     | 32  | Oligoarthritis        | 24  | 8              | 0                | 6                     |

Cellular Immunity and Amyloid

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While there is circumstantial evidence implicating immunologic dysfunction in amyloid disease, most data have precluded an etiologic role for circulating antibody and none is available concerning the role of delayed hypersensitivity. Therefore, to assess directly cellular immune function in the genesis of amyloidosis the following experiments were performed. Twelve Hartley guinea pigs were sensitized simultaneously to diphtheria (Dpd) and casein in complete Freund's adjuvant by footpad injection, then tested by the in vitro macrophage inhibition test of David et al. Macrophage inhibition of greater than 60% to both antigens (p < 0.01) developed within 1 week and persisted for 4 weeks. A second series of animals were similarly sensitized to both antigens but also received 1 ml 10% casein subcutaneously, 3 times per week for periods varying from 1 to 4 weeks. These showed significant macrophage inhibition to Dpd by Week 1 and to casein by Week 2. However, by Week 4 macrophage inhibition to both Dpd and casein was completely abolished. Finally, 24 guinea pigs were given multiple subcutaneous casein injections for periods varying between 8 and 52 weeks prior to footpad immunization with Dpd and casein. Groups of 4 animals were sacrificed bimonthly for tissue examination by congo red staining, and for evaluation of their response to Dpd and casein by macrophage inhibition. This last regimen produced increasing amounts of amyloid in the tissues at Week 16 and thereafter. It was also found that unresponsiveness to casein persisted throughout the entire experimental period, whereas sensitivity to Dpd was not inhibited. These experiments provide the first clear evidence that impaired cellular immunity is present during the induction of amyloidosis and may contribute to its pathogenesis.

Albumin Amino Acids in Patients with Rheumatoid Arthritis

CHARLES W. DENKO, Cleveland, Ohio, D. BARRIE PURSER, East Lansing, Michigan and RALPH M. JOHNSON, Logan, Utah

The metabolic processes operative in patients with rheumatoid arthritis are known to cause profound changes in metabolism of proteins. These effects are demonstrated clinically by wasting of

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muscle, skin and bone. To delineate further the extent of biochemical abnormalities in patients with rheumatoid arthritis, we determined the amino acid composition of an easily accessible structural protein, albumin.

Amino acid determinations were done in an automated amino acid analyzer by ion-exchange chromatography. Serum albumin from 30 patients with rheumatoid arthritis was compared in amino acid composition to serum albumin from 24 normal individuals. Homogeneity of albumin was established by polyacrylamide gel electrophoresis. A human albumin preparation, crystallized 4 times, was analyzed repeatedly by the same technics to serve as the reference standard. The percentage distributions of amino acids in the unknown albumin were then expressed as ratios of the percentage distribution of the amino acids when compared to the amino acids derived from a standard albumin sample.

The amino acid composition of the albumin from patients with rheumatoid arthritis differed from the composition of albumin from normal adults in the levels of phenylalanine and lysine. The changes were of similar magnitude, phenylalanine being about 14% greater (arthritics, 0.91; normals, 0.80; p < 0.02) and lysine about 14% lower, (arthritics, 0.85; normals, 0.99; p < 0.01). Such differences in composition of structural proteins in patients with rheumatoid arthritis have not previously been demonstrated.

It is not known whether or not these changes are the result of rheumatoid arthritis. There is little evidence in the literature to support the idea that substitution of one amino acid by another may occur by chance during protein synthesis.

A Screening Technic for Biochemical Abnormalities in Degenerative Cartilage Disease

HERBERT DIAMOND, DAVID HALBERSTAM and DAVID KAPLAN, Brooklyn, New York

Osteoarthritis, primary, or secondary to heritable or acquired disease of cartilage, may be due to a structural abnormality of mucopolysaccharide (MPS)-protein complex, increased activity of degradative systems, or lack of normal inhibition of such systems. A procedure to screen cartilage biopsy samples and serum for such defects has been devised.

Twenty milligrams of lyophilized fresh human cartilage are incubated in 1 ml of physiologic buffer, and the supernatant fluid analyzed for uronic acid, protein, hydroxyproline and molecular size by gel filtration. Following a rapid extraction phase of 1 hr, there is further release of MPS-protein at a linear rate during the next 24 hr reproducible for any individual cartilage specimen. Release was maximal at pH 7.4 and was inhibited by EDTA 1 mg/ml, iodoacetate, preheating cartilage for 15 min at 80° C and by 0.5 ml of fresh normal human serum. Release was not inhibited by albumin, γ globulin, haptoglobin or serum heated to 63° C for 45 min. Serum inhibition was retained after dialysis.

Eight cartilage specimens have been studied in terms of release of nondialyzable uronic acid and protein. In subjects with no clinical evidence of cartilage disease, uronic acid release at 24 hr was less in 4 subjects, ages 40-50 (0.31 mg/100 mg cartilage) than in 3 subjects, ages 14-25 (0.55 mg/100 mg cartilage).

Inhibition of extraction has been demonstrated in all 50 sera studied, including 6 normals, 7 patients with osteoarthritis, 12 rheumatoid arthritics, and 4 gouty subjects.

This procedure can be employed to detect abnormalities of MPS-protein release, characteristics of the released material or inhibition of release by serum.

Effect of D-Penicillamine on Glycosaminoglycan Changes During Rabbit Tendon Regeneration

ROBERT W. DORNER, Saint Louis, Missouri

Penicillamine is known to inhibit collagen cross-linking. This property was used to study the temporal patterns of change of glycosaminoglycan composition during rabbit tendon regeneration under conditions precluding collagen maturation.

Rabbit Achilles tendons were removed surgically and regeneration allowed to take place for periods of 4 days to 4 months while the animals received 70 mg/kg of D-penicillamine daily by subcutaneous injection. Regeneration tissue was collected and analyzed for collagen solubility, hexosamine/hydroxyproline ratio, glycosaminoglycan composition
(CPC column procedures), and solubility profile of the CPC complexes. The results were compared with previously published control values. Collagen solubility of the regeneration tissue was generally increased two-fourfold over control values. Hexosamine/hydroxyproline ratios of treated and control tissues followed similar patterns of decrease over the 4-month period. Patterns of change of glycosaminoglycan composition were generally similar in control and penicillamine-treated regeneration tissues except for a systematic departure in the dermatan sulfate fraction during the last 3 months of regeneration. At 28 days of regeneration the dermatan sulfate fractions of both treated and control tissues accounted for about 40% of the glycosaminoglycan hexosamine. Whereas the dermatan sulfate fraction of control tissue steadily rose to 54% over the ensuing 3 months, this fraction from treated tissues remained constant. In mature tendon from penicillamine treated rabbits dermatan sulfate fraction was reduced to 48% (control 68%) of total.

That dermatan sulfate is usually associated in connective tissues with mature insoluble collagen has been recognized previously by Loewi and Meyer. The present study demonstrates this association in a controlled experimental system.

**Mechanism of Lysosomal Hydrolase Release from Phagocytic Cells**

**PETER DUKOR, ROBERT B. ZURIER and GERALD WEISSMANN, New York, New York**

Damage to connective tissue may be mediated by lysosomal hydrolases extruded from phagocytic cells. To study mechanisms of enzyme release during phagocytosis in vitro, purified mouse peritoneal macrophages and polymorphonuclear leucocytes from human blood were exposed to various particulates (zymosan, latex, opsonized sheep red blood cells, heat-precipitated BSA). Uptake of undigestible, but not digestible, materials was associated with release (up to 15% in 2 hr from macrophages, up to 25% in 30 min from polymorphs) of lysosomal enzymes (β-glucuronidase, aroylsulfatase, acid phosphatase and acid protease). There was no release of the cytoplasmic enzyme lactic dehydrogenase during phagocytosis, although this enzyme was readily released from cultures by freezing or mild sonication. Selective extrusion of lysosomal hydrolases was quantitatively related both to the time of phagocytic exposure and to the number of ingested particles. Hydrolase release did not depend on loss of cellular integrity as judged by dye exclusion or by viability of macrophages in long-term cultures. Stabilizers of isolated lysosomes (chloroquine, hydrocortisone $10^{-4}$ to $10^{-5}$ M) failed to inhibit enzyme release or particle uptake. Agents which interfere with microtubular functions (colchicine, vinblastine $10^{-5}$ to $10^{-4}$ M) retarded both hydrolase extrusion and phagocytosis. Cyclic nucleotides ($3', 5'$ cyclic adenosine monophosphate, $3', 5'$ dibutyryl-cyclic AMP, $2', 3'$ cyclic AMP, $3', 5'$ cyclic guanosine monophosphate; $10^{-4}$ to $5 \times 10^{-4}$ M) inhibited hydrolase release but not particle uptake, while $5'$ AMP and EDTA ($5 \times 10^{-4}$ M) blocked both hydrolase extrusion and particle ingestion. Adenosine (up to $5 \times 10^{-4}$ M) was ineffective. Data suggest that uptake of undigestible materials leads to release of lytic enzymes from phagocytes by a process unrelated to general membrane damage. Enzyme release and phagocytosis can be modified by nucleotides and microtubule reagents.

**Digital Computer Analysis of the Renal Catabolism of L-Chain Protein as a Measure of the Renal Injury of SLE**

**WALLACE V. EPSTEIN, MARGARET TAN and HUGO M. MARTINEZ, San Francisco, California**

In our experience, increased urine concentrations of free light (L) polypeptide chains of the immunoglobulins occur early in the course of systemic lupus erythematosus (SLE) and reflect the renal lesion rather than the activity of the disease in general. Since normally L-chain protein is removed from the circulation almost entirely by renal catabolism (80–90%) rather than by excretion, delayed disappearance of $1^{14}C$ labeled normal L-chain protein from the intravascular space can be taken as a measure of the renal lesion of SLE. The turnover rate of L-chain protein is too rapid to be studied by ordinary graphical methods. Therefore a two-compartment mathematical model was developed and a digital computer program written for least-squares exponential curve fitting. This measure of renal catabolic capability is expressed as liters of plasma cleared per hour. Four normal subjects showed an L-chain catabolism of $1.70 \pm 0.22$ (SD) liters/hr while four studies in 3 anephric subjects showed an extra-renal catabolic capability $0.429 \pm \ldots$
0.03 liters/hr. Seven patients with renal biopsy-proven SLE were studied. Six of the 7 had elevated urine concentrations of endogenous L-chain protein (40 to 2,560 μg/ml, N < 20) and 2 of the 7 had increased serum L-chain concentrations. These latter 2 had the lowest Ccr of the group, 56 and 66 ml/min. Despite the relatively good renal function of this group the renal capability for catabolism of 125I labeled L-chain was 1.00 ± 0.22 liters/hr, a figure significantly below that of the normal controls. Measurement of the renal catabolic capability for L-chain protein provides a measure of the quantity and quality of the renal lesion of SLE prior to loss of renal excretion capacity.

Impaired Lymphocyte Responses in Ankylosing Spondylitis

FEMA ESCANILLA, F. PAUL ALEPA and WILLIAM REEFE, Washington, DC

The incorporation of tritiated thymidine by cultured lymphocytes in response to phytohemagglutinin (PHA), purified protein derivative (PPD) and mumps antigen was measured in 18 patients with definite ankylosing spondylitis. Skin responses to PPD, mumps and 2, 4 dinitrochlorobenzene (DNCB) sensitization were also studied.

Nine of the 18 patients (50%) had depressed lymphocyte responses to PHA of 3 SD or greater as compared to a control group of normal males. This is twice the reported incidence in rheumatoid arthritis and contrasts with the normal responses observed in Reiter's syndrome. The patients were divided into clinically active (8) and inactive (10) groups. Their ages ranged from 34 to 49 years. Twelve took daily phenylbutazone (200-400 mg). Five had received radiotherapy. The PHA response did not correlate with age, duration of illness or previous radiotherapy. Six of the 8 (75%) with clinically active disease had a depressed PHA response in contrast to 3 of the 10 (30%) in the inactive group (p = 0.05). All 9 patients with depressed PHA responsiveness were taking phenylbutazone (p = <0.01). However, 6 patients with Reiter's syndrome or gout receiving daily phenylbutazone (200-400 mg) had normal PHA responses.

Nine of the 18 had positive skin tests with PPD and/or mumps. The in vitro lymphocyte response to these antigens was concordant with the skin tests in 34 of the 36 measurements (94%). In contrast, PHA responses did not correlate with the skin tests or the in vitro lymphocyte responses to these antigens. The dissociation between the response of lymphocytes to PHA and to antigens has been reported in certain viral infections. DNCB sensitization occurred in all patients tested regardless of the other findings.

The Natural History of Systemic Lupus Erythematosus by Prospective Analysis

DOROTHY ESTES and CHARLES L. CHRISTIAN, New York, New York

A prospective study on the natural history of systemic lupus erythematosus (SLE) involving 150 patients has been in progress for the past 5 years. Data has been computer programmed and kept current by annual personal interview. Past information on patients under care when the study began was taken from hospital charts.

The clinical, hematologic and serologic manifestations in this study have been analyzed with respect to mode of onset, course and prognosis. The age at onset, recorded as appearance of multisystem disease rather than as time of diagnosis, occurred most frequently (92%) in the second decade of life. Six percent of patients presented with nephritis; 53% eventually developed nephritis, and half of the latter became nephrotic.

The 5-year survival rate for the entire series as calculated by the method of Merrill and Shulman (J Chronic Dis 1:1, 1955) was 75%. Sex, race and age at onset had no significant effect on this percentage. Clinical manifestations in patients with rheumatoid factor were the same as those in the entire series as was the 5-year survival rate (74.2%). Survival rates calculated from the time of recognition of specific organ system involvement showed the worst prognosis with the development of diffuse cerebral vasculitis. None of the 9 patients survived 5 years. The 5-year survival after the onset of lupus nephritis was also poor (50%) and correlated with pathologic findings on renal biopsy: 62% for 13 patients with focal glomerulonephritis and 26% for 7 patients with diffuse proliferative glomerulonephritis. However, the overall survival of this series showed significant improvement when compared with past series.
Patients receiving immunosuppressive therapy were too few to provide statistically significant data with regard to survival. However, this study should serve as a basis for evaluation of future therapies.

**Indications for Surgery in Juvenile Rheumatoid Arthritis**

**Edward J. Eyring and Jack C. Bass, Columbus, Ohio**

Review of our Arthritis Clinic material and that reported by others indicates that approximately half of the children with JRA will have significant disability in at least one joint. During the past 3 years, 29 of our 105 patients have undergone at least one orthopedic procedure for relief of pain, contracture, radiologic deterioration or limitation of motion. All of the patients are improved functionally, although every procedure has not been an unqualified success. We have performed 87 synovectomies on 85 joints, 20 tenotomies, 18 joint reconstructions and 16 manipulations. An aggressive therapy program has been begun postoperatively, supervised by the entire clinic team.

Forty-eight synovectomies have been followed more than 2 years. No motion has been lost in the legs, though wrists and fingers often lose motion which is compensated for by improved alignment. Two joints have come to repeat synovectomy. Radiologic improvement has persisted in 30% of joints. We have had no serious complications and have noted that most patients experience transient general improvement for several days to 6 weeks postoperatively.

Reconstructive procedures, including cup arthroplasties, have been successful uniformly within the limits of our preoperative goals.

**Human Blood Fractions and Aspirin Hydrolysis**

**Edward J. Eyring, A. John Merola and Maryann Jurkowitz, Columbus, Ohio**

It has been shown that acetylsalicylate (ASA) acetylates albumin under certain conditions in vitro. The purpose of this study was to see if hydrolysis of ASA itself is dependent on acetylation.

Plasma leucocytes, erythrocytes and erythrocyte membranes were incubated at 37°C with saturating concentrations of the substrate, ASA. Initial velocities were calculated as micrograms ASA hydrolyzed per milliliters of initial solution per minute.

Preparations exhibited the following hydrolytic activity: whole blood 0.60; erythrocytes 0.53; plasma 0.20; erythrocyte ghosts 0.07 and leukocytes < 0.01. These data indicate that the major blood activity is present in the erythrocyte contents. Bovine serum albumin inhibited hydrolysis by intact erythrocytes. This inhibition was concentration-dependent. Inhibition of ASA hydrolysis was itself counteracted by acetylation of the albumin. These data indicate that hydrolysis of ASA is not dependent in acetylation of albumin.

**Intracellular Phosphoribosylpyrophosphate (PRPP) Depletion by Allopurinol in Man**

**Irving H. Fox and William N. Kelley, Durham, North Carolina**

In addition to its inhibitory effect on xanthine oxidase, allopurinol inhibits the de novo synthesis of purines. Phosphoribosylpyrophosphate (PRPP), an essential substrate for the initial and rate-limiting step of purine biosynthesis, has been assayed in human erythrocytes by a method involving the conversion of labeled adenine to its nucleotide in the presence of partially purified adenine phosphoribosyltransferase enzyme. We have found: (1) The normal concentration of PRPP in erythrocytes ranged from 1 to 4 \times 10^{-6} \text{ M} which is substantially less than the known \( K_m \) values for this substrate. (2) Allopurinol but not oxipurinol consumed intracellular PRPP in vitro at a concentration as low as \( 2 \times 10^{-6} \text{ M} \) by a mechanism dependent on the presence of hypoxanthine-guanine phosphoribosyltransferase (PRT). (3) Allopurinol was converted to its ribonucleotide in vitro. (4) The administration of allopurinol in a single dose ranging from 2.2-4.0 mg/kg to 9 patients with normal PRT activity produced a significant decrease in erythrocyte PRPP content. The maximum decrease occurred 3-5 hr after administration of the drug, and the values observed ranged from 36-76% of control values with a mean of 47%. This effect preceded any significant change in the plasma urate concentration.
tration and urinary uric acid and oxypurine excretion. (5) Oxipurinol (4.0-8.0 mg/kg), the major product of allopurinol metabolism, had no significant effect on erythrocyte PRPP content after its in vivo administration.

The observation that the effects of allopurinol include depletion of PRPP and formation of allo-

**Steroid Treatment of Takayasu's Arteritis**

ANTONIO FRAGA, GREGORIO MINTZ, LORENZO VALLE and GILBERTO FLORES I, Mexico City, Mexico

The clinical picture of Takayasu's arteritis has been extensively reported. Treatment of this condition is varied and no conclusive, long-term results or survival data are available. The purpose of this investigation is to report our clinical experience in 22 patients with Takayasu's disease and the therapeutic results in 12 of them.

The criteria for inclusion in this series were: (1) decreased arterial blood flow, abnormalities of peripheral pulses, changes in blood pressure, and abnormal oscillometry; (2) abnormal angiogram (Seldinger or Steinberg technics); (3) biopsy; (4) autopsy; (5) chest x-ray.

Five patients died early in the course. In 2 of them, the diagnosis was established at autopsy. Two other patients had contraindication for steroid treatment: 1 with milliary tuberculosis, 1 with severe hypertension; 3 patients were lost to follow-up. The results were evaluated after a mean of 24.5 months of treatment (7-49 months) in this open, uncontrolled study.

Prednisone was started in 12 patients at a 30 mg/day dose for 2 months and then tapered according to clinical and laboratory data. The following changes were observed when comparing pretreatment and post-treatment evaluation: disappearance of headache in 5/11, muscular weakness 7/9, pain 5/8, paresthesias 3/9, blurred vision 2/5, dizziness 2/7, Raynaud's phenomenon 2/2, tinnitus 2/2, dyspnea 1/2. In 3 patients, pulses reappeared in 7 arteries and improved in 2 other arteries. Sedimentation rate improved at a significant level in all of them. None of the parameters worsened during the observation period, and the 4 patients without treatment (2 died and 2 with contraindications) showed extension and worsening of the disease.

It is concluded that steroid treatment has a clear effect reverting general symptoms and decreasing sedimentation rate, and can produce reappearance or improvement of the peripheral pulses in Takayasu's disease.

**The Scallop Sign: A Roentgenographic Finding in Rheumatoid Arthritics with Ruptured Finger Extensor Tendons**

RICHARD A. FRIEBERG and AARON S. WEINSTEIN, Cincinnati, Ohio

Thirteen cases of extensor tendon rupture in the fingers of patients with classical rheumatoid arthritis have been reviewed. A study of the hand and wrist roentgenograms of these patients, all women, revealed a common finding in addition to other radiographic changes of severe rheumatoid arthritis. This common finding, a large, easily identified erosion in the medial aspect of the distal radius, we have termed the "scallop sign." Erosion and destruction of the distal ulna are also invariably seen and may actually be the lesion which causes the extensor tendon ruptures by mechanical abrasion of the tendons. In no case, however, did extensor tendon rupture occur until the local synovial disease had become so invasive and destructive that a scallop sign appeared.

Prompt corrective surgery was performed in each instance. The surgical findings and management is discussed.

An association of a radiographic finding with finger extensor tendon ruptures is described. Now, because of our experience, when the scallop sign is seen prior to extensor tendon rupture, prophylactic surgery is advised.

**A Controlled Trial of Cyclophosphamide Therapy in Connective Tissue Disease**

JAMES F. FRIES, GORDON C. SHARP, HUGH O. McDEVITT and HALSTED R. HOLMAN, Stanford, California

Cytotoxic and immunosuppressive therapies have been increasingly employed in the management of patients with connective tissue diseases; few controlled studies of the usefulness of these agents are
available. This study compares cyclophosphamide, given alone, with prednisone alone, in the treatment of connective tissue disease. Evaluation was performed on 22 patients, assigned randomly to one of the two treatment groups. Fourteen patients with systemic lupus erythematosus (SLE) and 7 with polymyositis were included. Patients declared a failure on one regimen were then placed in the other group. Initial clinical and laboratory status of patients in both groups was comparable. In the cyclophosphamide group, no responses to therapy and 12 failures were observed. In the prednisone group, 14 responses and 5 failures were recorded.

Side effects were distressingly frequent in both groups. In cyclophosphamide-treated patients a 20% incidence of ovarian failure was found, correlating well with the absence of ovarian follicular structures found in mice treated with long-term cyclophosphamide therapy, and found also in 1 patient at autopsy.

Cyclophosphamide, as the sole agent, is significantly less useful than prednisone in the treatment of both SLE and polymyositis. The difference is most marked with regard to nonrenal manifestations. The failure to observe beneficial effects with cyclophosphamide may have been related to several factors. In 3 patients, severe side effects necessitated termination of treatment before 8 weeks. In 3, failure to control severe nonrenal inflammatory manifestations required transfer to the other treatment group. In the other 6, failure to respond after 2 to 4 months of therapy was noted. Special problems arose with the administration of a leukopenia-producing drug in a disease characterized by leukopenia. The possible role of cyclophosphamide in combination with prednisone remains to be defined.

**Diminished Fibrinolytic Activity and Massive Thrombotic Arteriopathy in Scleroderma**

Nancy L. Furey, Ha J. Kwaan, Nibha Suwanwela and Frank R. Schmid, Chicago, Illinois

Despite absence of demonstrable blood clotting abnormalities, repeated massive thromboses of small and medium-sized arteries have been observed in 4 women with scleroderma and Raynaud's phenomenon. Occlusions of mesenteric, femoral and digital arteries led to death in 3; amputation of digits occurred in the fourth patient. Widespread and marked thickening of arterial intima with fibrosis and recent thrombus formation was observed in vessels supplying gangrenous tissue.

Using the histochemical fibrin slide technic on tissue obtained at autopsy or surgery, active fibrinolytic activity was found in nonoccluded arteries with intimal thickening in 2 patients, whereas no such activity was found in similar but obstructed vessels at the site of thrombus. In a third patient, thickened arteries showed similar fibrinolytic activity, but the thrombotic arteries were not studied.

These findings are in direct contrast to the expected marked fibrinolytic activity in endothelium surrounding thrombi in a variety of other states, viz, coronary artery atherosclerosis, disseminated intravascular clotting, venous thrombosis and pulmonary artery embolism. An absence of fibrinolytic activity in thrombosed vessels has been reported in only one other condition—thrombotic thrombocytopenic purpura. The finding of local absence of fibrinolytic activity in thrombosed arteries in scleroderma suggests that both events may be closely interrelated. Whether such changes occur more generally in scleroderma or only in selected patients with major thrombotic episodes remains to be investigated.

**Bacterial and Mycotic Infections in Systemic Lupus Erythematosus**

Dale N. Gerding, Parker J. Staples, Robert S. Gordon and John L. Decker, Bethesda, Maryland

The course of 54 patients under observation in hospital for a total of 5294 days was analyzed retrospectively using automated technics. There were 23 with systemic lupus erythematosus (SLE), 20 with rheumatoid arthritis (RA), and 11 with idiopathic nephrotic syndrome (NPS). The latter 31 patients (NON-SLE) were selected randomly with regard to infection but with preference for those on adrenal corticosteroid therapy. Cytotoxic agents were used in 3 patients with SLE and 5 with NPS.

Thirty-nine episodes of bacterial or mycotic infections were identified in the course of intensive surveillance including 694 cultures. The infections covered a wide range of sites and organisms; of note were 6 urinary tract infections with E. coli and 5 episodes of β-hemolytic streptococcus septicemia. The infection rate (IR) (episodes of infection per Arthritis and Rheumatism, Vol. 13, No. 3 (May-June 1970)
100 days of observation) was 1.64 for SLE and 0.16 for NON-SLE (0.0 for RA and 0.23 for NPS).

In SLE, IR increased in proportion to dosage of adrenal corticosteroid therapy (prednisone equivalents, mg/day): none, 0.43; 1–20 mg, 0.92; 21–50 mg, 2.17; 51–80 mg, 2.12; and >80 mg, 4.00. At all dose levels, IR in SLE substantially exceeded IR in NPS.

The patients were scored for evidence of disease activity. The IR increased as the score increased both in SLE and NPS. No relationship could be found between IR and several parameters of renal disease except that azotemia (BUN >60 mg%), observed in SLE on 248 days and NPS on 43 days, was associated with an IR of 3.2 in SLE and 0 in NPS.

It is concluded that infections are more common in SLE than in RA or NPS, that adrenal corticosteroid therapy increases the risk but does not account for the prevalence, and that the risk is greatest in SLE patients with findings of active disease or with azotemia.

The Relationship of Lowered Complement (C') Activity and Macroglobulin Electrophoretic Mobility in Sera of Patients with Waldenstrom's Macroglobulinemia (W Macro)

M. Glovsky and H. Hugh Fudenberg, Los Angeles and San Francisco, California

Immunoglobulin binding to and inactivation of complement components has provided intriguing models in the study of vasculitis and arthritis. Recently we have noted low whole serum C' and C'2 activity in the sera of patients with (W Macro). Associated with the lowered whole C' activity was a more rapid electrophoretic migration of the para-protein, than that found in sera of normal complementemic patients. Also present, but not directly related to macroglobulin motility, was lowered C'1 and C'3 activity in at least 50% of the sera studied.

No correlation between lowered C' activity, macroglobulin content, antinuclear antibodies, cryoglobulins, cold agglutins, or anti-γ globulins could be found.

An apparent relationship between disease symptoms and whole C' activity in patients with W macro is of great interest. Diminished complement activity was associated with relative absence of disease symptoms. Three patients observed with low whole complement had no signs of the hyperviscosity syndrome and anemia and fatigue were not prevalent. Among the 9 normocomplementemic patients, 4 had symptoms of hyperviscosity, and 2 were anemic and required frequent transfusions. It is theoretically possible that under these circumstances complement exerts a protective effect. Such protection could occur either by promoting the destruction of lymphocytes important in the production of macroglobulins, or by the removal of antigenic substances related to macroglobulin formation.

It is suggested that certain classes of macroglobulin paraproteins may, because of either specific structural configuration or binding to other serum proteins, possess differences in both mobility and complement inactivation, as well as pathologic mechanisms.

Polyarthritis and the Australia Antigen: A New Association

David J. Gocke, Konrad Hsu, Councilman Morgan, Stefano Bombardieri, Michael Lockshin and Charles L. Christian, New York, New York

Four patients have been observed with polyarteritis associated with Australian antigen (AA). A diagnosis of polyarteritis was based in each on typical vascular lesions in muscle and liver. AA was detected in the serum of all 4. The best studied case occurred in a 48-year-old female who received a transfusion of blood containing AA during a routine operation. Three weeks later she returned because of fever (104°F), and AA had appeared in her serum. Over the next 2 months, the fever persisted, progressive hepatic enzyme abnormalities developed, and ulnar and peroneal neuropathies appeared. Liver biopsy revealed active hepatitis and inflammatory changes in arterioles characteristic of polyarteritis. Muscle biopsy confirmed the diagnosis of polyarteritis. At 3 months, an acute renal crisis occurred with hypertension, pulmonary edema, hematuria and azotemia. Steroid therapy resulted in defervescence of fever and improvement in hepatic function. However, hypertension, renal and hepatic abnormalities and AA are still present 9 months after the transfusion.
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The titer of AA rose when the patient was relatively well and declined during periods of exacerbation. Serum complement levels were depressed only during the renal crisis. The patient's serum was examined for immune complexes by zonal centrifugation (35-50% sucrose, 157,000 × g for 15 hr). Pellet fractions were AA negative by immunodiffusion. However, electron microscopic examination of negatively stained suspensions of the pellets revealed particles characteristic of those described in association with AA. Immunofluorescence studies of muscle carried out with fluorescein-labeled anti-AA, anti-IgM, and anti-B1c antisera revealed specific fluorescent deposits in the walls of small blood vessels. Control specimens were negative. Similar findings were made in the other patients. These observations suggest that the diffuse vasculitis seen in these patients was caused by deposition of complexes of AA, immunoglobulin and complement in small blood vessels. Current evidence indicates that AA is intimately associated with a virus which is one of the etiologic agents of hepatitis. This may be the first recognition of a systemic vascular disease in humans mediated by an immunologic reaction to a viral agent.

Quantitation of Gastrointestinal Bleeding and Therapeutic Effectiveness of Choline Salicylate Compared to Aspirin in Rheumatoid Arthritis

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Choline salicylate and aspirin in tablets that supplied equimolar salicylate concentrations were administered to 26 outpatients with definite or classic rheumatoid arthritis. Patients were examined weekly for 4 weeks in a double-blind, cross-over study to determine the gastrointestinal toxicity and therapeutic effectiveness of the compounds. During the first 2 weeks, they took tablets of 1 of the compounds, selected at random, and during the last 2 weeks, equal numbers of tablets of the other, verified by counts of returning medication. Amounts of salicylate previously found to be optimal for patient comfort were used (mean value, 3.6 g aspirin/day). Except for 6 patients on maintenance gold therapy, no other antirheumatic drugs were used.

Statistical analysis of the results showed no difference in therapeutic effectiveness of the 2 salicylate compounds as determined by the following parameters: duration of morning stiffness, grip strength, joint swelling (ring sizes), time to complete a measured walk and subjective pain severity. Gastrointestinal toxicity was assessed from history of gastrointestinal symptoms and by quantitation of stool blood loss. Equal numbers of patients (10) complained of nausea, stomach upset or diarrhea while receiving either aspirin or choline salicylate. Patients' erythrocytes were tagged with radioactive chromium, and stool specimens were collected during the last 4 days on each salicylate compound. Excellent patient reliability was confirmed indirectly by the striking uniformity of the results. Except for 1 patient on the aspirin-choline salicylate cross-over and 2 on the reverse cross-over, a consistent pattern of greater bleeding on aspirin was demonstrated (mean blood loss per patient for 4 days on aspirin 23 ml; on choline salicylate, 8 ml; p < 0.01).

Gastrointestinal toxicity is a major complication of salicylate therapy and frequently hinders treatment of rheumatoid patients. Choline salicylate in tablet form appeared to be as effective as aspirin in suppressing the clinical manifestations of rheumatoid arthritis while producing significantly less stool blood loss.

The Extra-Articular Features of Rheumatoid Arthritis

DUNCAN A. GORDON, JACK L. STEIN, DAVID A. BELL and IRVIN BRODER, Toronto, Canada

The possibility was considered that patients with rheumatoid arthritis who exhibit extra-articular features (EAF) may have a different disease than those without EAF.

One hundred and twenty-seven hospitalized patients with definite or classic rheumatoid arthritis were prospectively examined, of whom 96 (76%) had EAF. The features considered as EAF were nodules, episcleritis, splenomegaly, lymphadenopathy, pericarditis, pleurisy, pulmonary fibrosis, skin ulceration, digital vasculitis and noncompressive neuropathy. The optimal contrast was obtained by comparing those patients without EAF (31) with those patients (46) who had both nodules and at least one additional feature.

The EAF group exhibited more advanced functional impairment, more severe clinical and radio-
logical articular destruction, and lower grip strength. The laboratory results demonstrated markedly higher titers of rheumatoid factor, a greater prevalence of LE cells, RBAF and antithyroid antibody, and greater elevation of immunoglobulins A and M. The EAF patients showed these same characteristics when matched with a non-EAF group of either short or long duration of disease. However, these differences were only relative and did not uniquely distinguish the EAF patients.

Therefore, it was concluded that the manifestations of rheumatoid disease are a continuum in which EAF merely represent one feature of more severe disease, unrelated to duration. This relationship of EAF to the other manifestations of rheumatoid disease has not been documented previously.

The Classification of Symptomatic and Functional Status in Osteoarthritis of the Knees
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The standardized radiographic criteria of Kellgren and Lawrence have provided the means for achieving diagnostic comparability and uniformity of morphological grading in population and clinical studies of osteoarthritis. However, an equivalent method for classifying symptomatic and functional status is lacking. The present study approached this problem in osteoarthritis of the knees. A literature review was done to classify the methods currently being used. A wide variety was found. The only variable included by all investigators was pain, and this was classified in many different ways. Meaningful indices of function were frequently absent. Problems presented by concurrent disease, disability and treatment were considered in less than half of the reports. Important descriptive characteristics of the patients studied were frequently omitted. No consistent methodologic approach was evident.

A systematic procedural format for performing such evaluations was then devised. This incorporates all standard variables. If followed systematically, it should provide an investigator or clinical observer with data which are both complete and comparable with the work of others. The time required is less than 15 min with a normal volunteer.

Additionally, tentative criteria for a ranked scale to permit graded classification of symptomatic and functional status in osteoarthritis of the knees are suggested. A subject is graded I to IV according to performance ability and accompanying symptoms in the progressive functional challenges of rising from a chair, walking and using stairs. A provision is made for identifying the presence of significant comorbidity. All of the information required by this graded classification system is obtained when the procedural format described above is used.

Tadpole Collagenase: Inhibition of Enzyme Activity by Antibody In Vitro
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At present there is widespread interest in the role of collagenases in the remodeling of tissue and in the mediation of tissue injury. The present report is one of a series of studies on the properties of tadpole tail fin collagenase, to date the most thoroughly characterized vertebrate collagenase.

Collagenase was isolated from culture medium in which living tail fin had been incubated for several days at 37° C. An enzymatically active fraction obtained by (NH₄)₂ SO₄ precipitation of the medium followed by agarose column chromatography showed a single band on disc gel electrophoresis. Rabbits were immunized with purified enzyme emulsified in complete Freund's adjuvant; antisera were obtained at intervals.

Normal rabbit serum, but not its γG fraction, inhibits amphibian collagenase. The γG fraction of specific antiserum was isolated by chromatography. Incubation of active enzyme preparations with this γG fraction resulted in inhibition of collagenolytic activity as measured by: (1) failure to reduce viscosity of guinea pig collagen solutions; (2) failure of release of 14C-glycine containing peptide fragments from guinea pig collagen or of collagen fragments as observed by disc electrophoresis; and 3) inhibition of lysis of collagen gels by living tail fin fragments. The γG fraction of anti-egg albumin antiserum had no inhibitory effect.

Absorption of the γG fraction with fresh tadpole tail fin extracts removed the inhibitory activity. In agar gel, a single precipitin line formed between purified enzyme preparations and the γG fraction of
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anti-collagenase antiserum. Three precipitin lines were observed with tissue extracts; one of these was antigenically related to the line formed by the enzyme preparations. Tissue extracts do not appear to have collagenolytic activity, and protein synthesis is required for active enzyme production.

The gel diffusion and absorption studies suggest the possibility that an inactive precursor of collagenase may exist in amphibian tissues which requires protein synthesis for activation. Attempts to demonstrate the existence of such a precursor directly by isolation and activation are in progress.

A Mechanism for Cartilage Destruction in Rheumatoid Arthritis

EDWARD D. HARRIS, JR, DONALD R. DiBONA and STEPHEN M. KRANE, Boston, Massachusetts

The unique morphologic and mechanical properties of articular cartilage result from the interrelationships of the components of the extracellular matrix. The destruction of this matrix is responsible for much of the deformity and dysfunction of the joint in rheumatoid arthritis. How is cartilage destroyed in RA? Phase and electron micrographs of the pannus/cartilage junction in a rheumatoid joint removed for insertion of a prosthesis revealed an advancing front of cells similar to those described as lining cells of the synovial membrane. Between these cells and normal cartilage a band was found which was depleted of collagen fibers, suggesting that extracellular degradation was taking place. There were no polymorphonuclear leukocytes or blood vessels at or near this junction of cartilage and pannus. A model of this invasive pannus of RA was produced by culturing rheumatoid synovium upon autologous tendon. After 5 weeks, explants were actively producing collagenase, as measured by assays using 14C-labeled collagen gels; electron micrographs (EM) showed synovial cells apparently invading collagen bundles.

Canine patella cartilage was used as a substrate to determine whether the collagenase produced and released by rheumatoid synovium in tissue culture was sufficient to destroy cartilage in the absence of enzymes which degrade proteinpolysaccharide (PPS). Trypsin, while depleting safranin 0 stain and metachromasia from cartilage (signifying loss of PPS), did not alter the gross appearance of cartilage; nor did it effect a release of hydroxyproline (HYPRO) into the media. On the other hand, partially purified synovial collagenase (with no demonstrable nonspecific proteolytic or hyaluronidase activity) dissolved the cartilage leaving a soft, transparent film; EM showed depletion of collagen fibers. By using cartilage with and without pre-incubation with trypsin as a substrate for collagenase it could be shown that PPS offered no protection to cartilage collagen from the action of collagenase.

These experiments suggest that a collagenase, presumably that derived from synovial tissues, can produce the destruction of cartilage seen in rheumatoid arthritis.

A Synovial Endopeptidase: Its Potential Role in Collagenolysis

EDWARD D. HARRIS, JR and STEPHEN M. KRANE, Boston, Massachusetts

Collagenase synthesized and released by rheumatoid synovium in culture cleaves collagen molecules into two fragments which, at 37° C, are denatured to gelatin and become susceptible to further breakdown. It is not known whether the collagenase itself or other proteases are involved in the subsequent degradation of the fragmented molecule. We report here characteristics of endopeptidase activity which is found in cultures of rheumatoid synovium and which does not degrade collagen in the native state but does degrade denatured collagen.

Rheumatoid synovial tissue was cultured at 37° C in Dulbecco’s modified Eagle’s medium (without serum). Medium harvested from Day 1 and 2 of culture was concentrated, and a 20-60% (NH₄)₂SO₄ fraction contained significant proteolytic activity as well as collagenolytic activity. A sensitive assay for proteolysis was developed using 14C-labeled gelatin as substrate: after incubation with proteolytic enzymes cold TCA was added (final concentration 15%); following centrifugation, radioactivity proportional to the proteolytic activity was demonstrated in the supernatant. Proteolytic activity was eluted before the collagenase from columns of Bio-Gel A-0.5 and Sephadex G-150. Protease activity versus gelatin was inhibited by EDTA and by reducing agents (dithiothreitol and cysteine), but was not inhibited by pCMB or soy bean trypsin inhibitor. There was no activity at pH < 6.0. When used in quantities which brought about equivalent degradation of gelatin, collagenase isolated from the same media was demonstrated to break down...
collagen in solution at an initial rate of 50 times that of the protease. Using an artificial substrate for bacterial collagenase (PBZ-pro-leu-gly-pro-D-arg), the protease cleaved the leu-gly bond which cannot be hydrolyzed by trypsin, chymotrypsin or exopeptidases.

Separation of enzyme which degrades the artificial substrate from enzyme which degrades native collagen has been reported in cultures from tadpole tissues (Harper and Gross: Biochim Biophys Acta 198:286, 1970). Apparently similar activity from synovial cultures as characterized here may function in the degradation of the large fragments produced by the initial cleavage of collagen by collagenase to small peptides which could be metabolized further or excreted in the urine.

**Suppression of the Immune Response to HGG In Mice by Rheumatoid Factor**

JOHN S. HECE and WALLACE V. EPSTEIN, San Francisco, California

The capacity of passively administered rheumatoid factor (RF) or 7S mouse anti-HGG antiserum to inhibit the responses of mice to immunization with HGG has been quantitated at the cellular level by the carbodiimide modification of the Jerne plaque-forming cell technic. This modification allows general application of the Jerne plaquing method to soluble protein antigens, providing for enumeration of cells forming antibody directed against a specific protein.

In this investigation, CAF₁ mice were immunized subcutaneously with 0.1 mg aggregated HGG in 0.05 ml Freund's incomplete adjuvant. The day after immunization, one group of mice received intraperitoneal mouse 7S anti-HGG antiserum. Another received a human IgM preparation containing high concentrations of rheumatoid factor. A third group was injected with a similar IgM preparation without detectable RF activity. A fourth group received no further treatment. Six days after immunization, numbers of spleen cells forming anti-HGG antibody were determined by the modified Jerne plaque assay. Mice receiving 7S mouse anti-HGG antisera had 86% fewer anti-HGG plaque-forming cells (HGG-PFC) than mice receiving no additional treatment (P < .005). Mice receiving human IgM with RF activity had 37% fewer HGG-PFC than those receiving IgM without detectable RF (P < .005). Control studies indicated the suppression was specific in both instances.

Rheumatoid factor lacked the capacity to initiate complement-mediated hemolysis of the Jerne HGG-coated indicator erythrocytes. Nevertheless, in vitro inhibition studies showed RF incapable of competitively inhibiting plaque formation by the hemolytically efficient 19S mouse anti-HGG antibody. These investigations suggest that RF and the mouse antibody do not compete for the same antigenic determinants. The suppression by intraperitoneally administered RF appears to be immunologically specific at the level of the antigen molecule, but not at the level of antigenic determinants.

**A Possible Role of Specific Anti-Antibody in Transplantation and in the Immune Disorders of the Connective Tissue**

NICOLAS RADOIU and FREDERICK A. ZYDECK, Detroit, Michigan

In recent years, the concept that antibody globulins show no immunologically recognizable differences from normal globulins has been refuted. Various investigators have demonstrated that anti-antibodies can be produced. It has been suggested that anti-antibody may be directed against the binding site of the antibody used as antigen. To investigate the potential use of such anti-antibodies in a therapeutic sense, we propose that it may be possible to train the individuals' lymphopoietic system to produce anti-antibodies against certain antibodies produced by the recipient in homograft rejection, or against antibodies present in certain immune diseases. To train the lymphopoietic system, we employed an immunologic triangle in which 3 animals were involved: a donor and a recipient of the same species, and an intermediate animal of a different species. The donor's tissue was used as an antigen to elicit antidonor antibody in the intermediate species. The antidonor antibody was then isolated and used as an antigen to elicit anti-antibody production in the eventual recipient. Such anti-antibody was intended to neutralize antibody produced by the recipient against the donor tissue. To demonstrate this, we chose a simple type of homograft model based on blood transfusion in the dog. Since the canine type A erythrocyte elicits potent hemolysins in dogs of a type other than A,
since dogs do not possess naturally occurring anti-A isoantibodies, this system provided a readily available particulate antigen, easily assayed in vitro. This paper presents results achieved in application of the proposed immunologic triangle to the training of the lymphopoietic system to produce anti-antibodies. Anti-antibody was produced and its action demonstrated using the immunologic triangle concept. The results achieved suggest that anti-anti-A neutralizes anti-A in vivo and prevents a demonstrable titer in hemolytic titration tests in vitro. The neutralization of anti-A by anti-anti-A has been shown to prevent transfusion reaction in recipients pre-treated for anti-anti-A production.

The Use of Gold in the Treatment of Juvenile Rheumatoid Arthritis (JRA)

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Approximately 20% of children with JRA develop a chronic progressively disabling arthritis which is unresponsive to the usual conservative methods of treatment. The use of corticosteroids in these children is dangerous, and the need for other forms of treatment is apparent. The object of this study was to review our experience in the treatment of JRA with gold sodium thiomalate over the last 10 years. Forty patients have been studied, 12 males and 28 females. The mean age at onset of JRA was 5 years, and the mean age at the start of treatment 8 years. The schedule of treatment was one injection per week of 1 mg/kg gold sodium thiomalate for 20 weeks, and then every 2 to 4 weeks for 2 years thereafter if there had been evidence of improvement. The mean duration of treatment was 21 months, and the mean number of injections given was 32. To insure the safety of the treatment, rigid criteria were used for the continuation of gold therapy. Therapy was discontinued in 8 of the patients due to a significant reduction in circulating granulocytes or proteinuria. Nineteen of 30 patients with systemic disease showed improvement in the systemic indices. Reduction of swelling was evident in 21 of 32, but limitation of motion decreased in only 10. Of 21 patients on steroid therapy at the beginning of treatment, 9 were able to discontinue steroid therapy entirely by the end of treatment and 10 were receiving 5 mg or less per day. Aspirin dosage was not significantly altered. Twenty-three of the children improved by at least one functional class, 8 by 2 functional classes. Seven of these went into complete remission. Possible gold toxicity was observed in 11 patients but was transitory. This study was not designed to distinguish between spontaneous or gold-induced improvement of JRA, but does suggest that more intensive study of the use of gold in the treatment of severe JRA is warranted.

Evidence for Altered Proteinpolysaccharide Complexes (PPC) in Fresh Osteoarthritic Cartilage in Human Hips

DAVID S. HOWELL, JULIO C. PITA, JUAN F. MARQUEZ, J. E. MADRUGA and FRANCISCO J. MULLER, Miami, Florida

Ultramicrobiochemical technics developed in this laboratory for isolation of whole PPC were applied to starting wet tissue samples of 5–10 mg and to micropuncture fluid samples of 20–60 ml. The PPC were prepared according to the methods of Sajdera and Hascall (J Biol Chem 244:77, 1969), as well as DiSalvo and Schubert (J Biol Chem 242:705, 1967). Hexuronate protein and phosphate were determined in the R1, R2 and S fractions. PPC were also subjected to dialysis and filtration according to a scaled-down method modified from that of Gerber and Schubert (Biopolymers 2:259, 1964). Articular cartilages obtained at surgery from patients with osteoarthritis, septic arthritis and gout, as well as nasal, articular and growth plate cartilages from rats, rabbits and cows were studied. The PPL R2 fraction of normal animal and human cartilages ranged from 23–31% of total PPC. The R2 fraction was absent in samples from “yellowish spots” with intact cartilage surface from severely degenerative lesions of human osteoarthritic hips and from purulent cartilages. Total PPC and hexuronate-to-protein ratio of PPC in osteoarthritic samples were low, and in contrast to “normal” cartilage from the same specimens, a small proportion of PPC hexuronate could be filtered through cellophane. R2 fractions consistently and effectively blocked our system of in vitro calcification, whereas R1 or S fractions consistently lacked this property. It is concluded that: (1) the R2 fraction is not an...
artifact of separation methods; (2) its absence is a sensitive indicator of altered macromolecular properties of PPC in cartilage; and (3) the current methods offer a new method for assay for degrada-
tive enzymes in cartilage. Results are suggestive of degradation of extracellular matrix proteoglycan-proteoglycans in these instances of osteoarthritis at an early histologic stage of the disease.

The Occurrence of DNA in Biologic Fluids

GRAHAM R. V. HUGHES, SELWYN A. COHEN, ROBERT W. LIGHTFOOT, JR and CHARLES L. CHRISTIAN, New York, New York

Previous studies by Tan et al have indicated that circulating DNA can be detected in some patients with systemic lupus erythematosus (SLE). Both Tan et al and Barnett have also reported the presence of free DNA in synovial fluid and in the serum of a few non-SLE patients. In this study, factors predisposing to the release of DNA into biologic fluids were sought. In particular, the relationship of DNA-release to corticosteroid therapy was studied.

Serum and synovial fluid samples were tested for the presence of DNA by immunodiffusion against a serum containing precipitating antibodies against native and heat-denatured DNA. The reactions were abolished by pretreatment with DNAse but not by pronase.

Serial sera were obtained from patients given high dose corticosteroid or immunosuppressives for a variety of diseases. In 2 of 5 SLE patients studied, circulating DNA was observed within 48 hr of initiation of high dose therapy. Of 15 patients with other diseases, 4 patients (2 with leukemia, 1 with dermatomyositis and 1 who received a renal transplant for chronic pyelonephritis) developed circulating DNA after starting therapy. One additional patient receiving cephalosporin for pneumococcal sepsis developed circulating DNA. Agar diffusion studies utilizing anti-DNA reagents with varying specificities suggested that the DNA in sera and synovial fluids was largely in the native form. In the 2 patients with SLE, anti-DNA antibodies (measured by gel precipitation and by the Farr technic) disappeared within 48 hr of initiation of therapy, suggesting that accelerated consumption of antibody had occurred.

Of 56 synovial fluids tested, from patients with a wide variety of disorders, 36 (64%) were found to have free DNA. There was a correlation between presence of DNA and synovial fluid lysozyme levels but none with diagnosis or synovial fluid cell counts.

Release of DNA may be a nonspecific sequel of cell breakdown. The release of DNA into the circulation, in response to therapy, or other events, may have pathogenetic implications in SLE where the presence of anti-DNA antibodies antedates the appearance of free DNA.

Virus Antibody Levels in Systemic Lupus Erythematosus (SLE)

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There has been interest recently in a possible viral etiology of SLE. Phillips and Christian have reported elevated antibody titers to the myxoviruses, measles and parainfluenza 1. In the current study, sera of 14 patients with well-documented SLE (with nephritis) and 14 matched normal controls were collected for antibody studies. Micro-complement fixation (CF) tests were performed for determination of antibodies to A2 soluble influenza; B soluble influenza; C influenza; parainfluenza 1, 2 and 3; mumps (soluble and viral antigens); measles and respiratory syncytial viruses (all RNA myxoviruses); infectious bronchitis virus OC43 (RNA coronavirus); and adenovirus and herpesvirus, both DNA viruses. Hemagglutination-inhibition (HI) tests were performed for determination of antibodies to parainfluenza 1, 2 and 3; mumps; influenza strains A/HK/8/68, A/JAP/170/62 and B/Mass/3/66; and measles (all RNA myxoviruses). Of the 13 viruses tested by the CF technic, 11 showed higher titers in SLE than in controls. In the remaining 2, the titers were essentially equal. The differences were significant in 5 instances: parainfluenza 1 (p < 0.02), respiratory syncytial (p < 0.02), infectious bronchitis OC43 (p < 0.05) and herpes (p < 0.05) viruses. Of the 8 viruses tested by the HI technic, 6 showed higher titers in the SLE group. The other 2 showed essentially the same titers as the controls. Differences were significant in 2 instances: parainfluenza 1 (p < 0.02) and measles (p < 0.05). The overall trend of increased antibody titer by the CF test in SLE was compared with controls and was highly significant (p < 0.000001); it was also significant by the HI

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Test (p < 0.002). These data demonstrate increased antibody titers in SLE to a group of RNA viruses (and in one instance a DNA virus). Previous data from this laboratory have not shown similar increased titers against bacterial antigens. The increased titers noted represent responses to diverse viral antigens—i.e., ribonucleoprotein, viral coat protein or host protein. Thus, they demonstrate increased antibody responsiveness to a variety of viral antigens in SLE.

Cytoplasmic Tubular Structures in Kidney Biopsies in Systemic Lupus Erythematosus (SLE) and in Patients with Various Renal Diseases
ERIC R. HURD, EDWIN EISENBRODT, STANLEY STRUNK and MORRIS ZIFF, Dallas, Texas

Recently myxovirus-like structures have been seen in renal biopsies and other tissues from patients with SLE. These structures were present in the endothelial cell cytoplasm of the glomeruli and consisted of interwoven tubular structures morphologically similar to nucleoprotein strands liberated from myxoviruses. Previous reports have implied a considerable specificity for SLE. In the current study we have examined a variety of renal biopsies in the electron microscope. Similar structures have been seen in all of 42 renal biopsies from 35 patients with SLE. Five of these were from patients with histologically normal kidney by light microscopy.

Renal biopsies from 115 random patients with renal disease who did not have SLE clinically were also screened. Twenty-four of these biopsies from 23 patients contained similar tubular structures in the endothelial cell cytoplasm, although the frequency of these structures was usually lower than in the SLE patients. Fourteen of the 23 patients were children (ages 3-10). Diagnoses of the 24 positive biopsies were as follows: acute diffuse glomerulonephritis, 9; chronic glomerulonephritis, 3; membranous glomerulonephritis, 1; focal glomerulitis, 5; and mixed cryoglobulinemia with acute glomerulonephritis, benign intermittent hematuria with normal renal biopsy, gold salt nephropathy in rheumatoid arthritis, sickle cell disease with nephritis, idiopathic nephrotic syndrome with normal renal biopsy, and eclampsia (normal histologic findings by light microscopy), 1 each. Careful examination of renal biopsies obtained from 8 normal volunteers showed no evidence of the structures.

Thus, while the structures appear to be uniformly present in SLE kidneys and in higher frequency than in other forms of renal disease, they are not specific for SLE. Details of the ultrastructure of the inclusions and their possible significance will be discussed.

Virtual Disappearance of M-Spike Globulin and Bence-Jones Proteinuria After Treatment with Melphalan
ROBERT IRBY, MARION WALLER and JOHN H. MOON, Richmond, Virginia

Zawadzki and Benedek recently reported the increased incidence of dysproteinemic and paraproteinemic diseases in patients with rheumatoid arthritis. They also mentioned disappearance of M-spike protein and bone reossification after treatment with melphalan, but no mention was made of change in other immunoglobulin or rheumatoid factor titers.

Our patient was a 62-year-old white male who had seropositive rheumatoid arthritis for 20 years. In 1966, the serum electrophoretic pattern showed an M spike in the y globulin region, and his urine showed Bence-Jones protein of the K-chain type. There were increased plasma cells in the marrow, and treatment with melphalan has been administered for the past 24 months. Other drugs include prednisone, salicylates and sodium fluoride. A recent serum electrophoresis showed virtual disappearance of the M spike protein as well as loss of Bence Jones proteinuria by heat test. Only after dialysis and repeated attempts was one able to demonstrate the slightest trace of abnormal protein on urine electrophoresis.

A comparison of the titers of his normal antiglobulin antibodies (IgG), as well as titers of rheumatoid factors (IgM) before and after treatment, showed little or no change. Clinically the patient's arthritis has improved remarkably and the steroid dosage has been halved. It is felt that melphalan may have some specificity in suppression of activity of the clone of cells producing this abnormal globulin.

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The Role of NZB/NZW Thymus and Bone Marrow in Cyclophosphamide-Induced Tolerance

MICHAEL E. JACOBS, JOYCE K. GORDON and NORMAN TALAL, Bethesda, Maryland

New Zealand mice spontaneously develop an autoimmune disorder and hyperrespond to certain antigens, including sheep erythrocytes (SRBC). An immunologic imbalance between thymus and bone marrow functions exists in these mice. We studied the ability of thymus and bone marrow cells from untreated and tolerant mice to act cooperatively to produce splenic plaque-forming cells (PFC) as determined by the Jerne technic.

Tolerance to SRBC was induced in 2-month-old NZB/NZW (B/W) and C57B1 mice by an intraperitoneal (IP) injection of \(5 \times 10^8\) SRBC, followed 24 hr later by cyclophosphamide, 100 mg/kg IP. This course was repeated in 5 days, and tolerance was maintained thereafter by injections of SRBC every 5 days. Thymus or marrow cells from tolerant (T) or untreated (U) mice were injected in various combinations into the syngeneic lethally irradiated host, which was later immunized with SRBC and assayed for PFC. The results are indicated in the Table below. Untreated marrow and thymus acted cooperatively to produce large numbers of PFC. Tolerant marrow and thymus produced few PFC, as expected. Tolerant B/W bone marrow, and tolerant C57B1 bone marrow and thymus, were unable to cooperate when combined with the appropriate untreated cell population. However, B/W thymus from tolerant mice could still cooperate with untreated B/W bone marrow, suggesting that this thymus could not express the donor animal's tolerance. Thus, in B/W mice, tolerance was transferred with the bone marrow, but not with the thymus. In C57B1 mice both cell types could transfer tolerance.

These results suggest that the B/W thymus may be relatively resistant to cyclophosphamide induced tolerance.

| Strain | Marrow | Thymus | PFC |
|--------|--------|--------|-----|
| Both   | U      | U      | ++++|
| Both   | T      | T      | +   |
| C57B1  | T      | U      | +   |
| C57B1  | U      | T      | +   |
| B/W    | U      | T      | ++++|

The Relation of Gold Pharmacodynamics to the Outcome of Gold Therapy in Rheumatoid Patients

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An attempt has been made to relate the outcome of gold therapy to pharmacodynamic studies of an intravenous Au\(^{198}\) thiglucose injection prior to treatment. Determination of tracer in the wrists, shoulders, knees, liver area and spleen area, as well as whole body counting was performed at intervals over a 2-week period following injection. Successful treatment in 3 rheumatoid subjects was related to an initially reduced uptake of the tracer by the liver, compared to a greater initial liver accumulation in two failures. Biologic half-life of the tracer was the same in these 5 cases, and also in a normal subject and a case of Reiter's syndrome in remission.

Liver entrapment of the tracer increased with time in the normal only, and slightly decreased in the others. No marked differences were noted in the uptake by individual joints, except for higher initial accumulation in inflamed joints, presumably due to increased blood supply.

A limited number of urine and fecal studies revealed a predominant excretion in the urine. The tracer was mainly associated with the albumin fraction of the plasma.

Progressive Systemic Sclerosis

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Twenty-eight patients with progressive systemic sclerosis (PSS) have been followed for periods of up to 3 years with repeated measurements of pulmonary diffusing capacity at 2 levels of oxygen breathing, quantitation of resistance of the skin to deformation, urinary hydroxyproline and 5-HIAA and blood serotonin.

Results: (1) There was no trend toward progres-
sive worsening of either pulmonary function or of skin abnormalities in these patients when studied at 4-6 month intervals. Seven deaths were all due to either acute exacerbations of disease, usually renal, or to superimposed pulmonary infection. Only 1 patient died with rapidly progressive pulmonary insufficiency. (2) In 17 pairs of pulmonary function tests, the first done during cold weather and the second done 4-6 months later during warmer weather, mean diffusing capacity rose from 14.3 to 18.2 ml CO/min/mmHg (p < 0.001), with 15 of 17 showing improvement. In 17 other pairs, where the first test was done during warm weather and the second during cold weather, diffusion changed from 14.3 to 13.1 ml CO/min/mmHg (p < 0.2), with 11 of 17 patients showing a decrease.

Conclusions: PSS may be a disease characterized by self-limited episodes of injury to small vessels and vasospasm. Such episodes may be fatal if they involve the kidney, or they may produce secondary fibrosis which is not necessarily progressive and does not indicate continuing activity of the underlying disease process. The pulmonary equivalent of Raynaud's phenomenon is demonstrable, and, as in the extremities, may or may not lead to irreversible changes in the lung, as seen in several patients who showed marked improvement in pulmonary diffusing capacity. The chemical tests did not provide useful information.
Induction of Antinuclear Antibodies (ANA), Rheumatoid Factor (RF) and C-Reactive Protein (CRP) in "Normal" Population Groups by Synthetic Estrogen-Progestogens

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Synthetic estrogen-progestogens used as oral contraceptives (OC) may alter serologic tests in patients with incipient rheumatic disease. Sera from 82 normal women 18 to 28 years of age were analyzed before treatment and after treatment with various OC for an average of 4 months. Development of positive tests for ANA (1:10 dilution) occurred in 4 (p < .05), RF (>1:40 titer) in 9 (p < .02), and CRP in 14 (p < .001). Six women had positive ANA and 3 positive RF before treatment with OC, and these tests remained positive during treatment. In women who developed positive ANA tests the OC contained >75 μg mestranol/tablet; frequency of RF increased with duration of therapy (>6 months). Mean IgG levels in the seropositive cases decreased during OC use from 9.6 ± 0.53 to 9.3 ± 0.41 mg/ml, while values in a comparable group of seronegative women matched for age, drug and duration of treatment decreased from 7.6 ± 0.45 to 7.2 ± 0.41 mg/ml (mean ± SEM). IgA levels were within normal limits in all but 1 woman. IgM levels were >2.3 mg/ml in 7 women: 3 developed ANA and 3 RF during treatment with OC. Sera from 174 women (mean age, 21) who had never taken OC demonstrated positive tests for ANA in 6.2%, RF in 4.6% and CRP in 14.3% of this group. In another group of 210 women (mean age, 25) who had been on OC for an average of 27 months, ANA tests were positive in 10%, RF in 7.7% and CRP in 23.3%. Only the increase in CRP was significant (p < .05) when comparisons of age, drug and duration of therapy were made between these last two groups. The incidence of positive tests for ANA in 130 OC nonusers (15–40 years of age) selected at random from 3000 participants in a Community Population Survey was 6% at 21 years and 5% at 25 years of age. These findings emphasize the importance of prospective studies when the effect of OC on individual serologic tests is investigated in different populations of apparently normal young women.

Induction of Lymphocyte Transformation by Synovial Fluid (SF) from Patients with Rheumatoid Arthritis (RA)

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In RA, lymphoid hyperplasia, follicle formation and synthesis of immunoglobulins and rheumatoid factor (RF) in the synovium are compatible with a local immune response. To ascertain whether RA-SF might participate in such a response, the in vitro transformation of RA and nonRA buffy coat lymphocytes cultured for 5 days with autologous and homologous SF was assessed morphologically and by scintillation counting for tritiated thymidine (H³T) uptake.

Freshly aspirated SF was centrifuged (4°C) for 1 hr at 49050 g, the upper half diluted 1:50, 1:100 and 1:200 in fetal calf serum (FCS) and inactivated once at 56°C X 30 min, 10⁶ RA or nonRA lymphocytes per tube were cultured in triplicate as follows: (1) 20% FCS (controls), (2) PHA, (3) autologous SF, (4) homologous nonRA-SF and (5) homologous RA-SF. All RA patients were positive and all non-RA patients negative for RF in serum and SF. To date, SF from nonRA patients with acute rheumatic fever (3), traumatic synovitis (2), osteoarthritis (2), and pseudogout (1) have been tested.

Morphologically, significant transformation (>5% above controls) was induced by 1:1 autologous RA, 3:5 homologous RA and 0:2 homologous nonRA fluids. By H³T uptake, RA-SF induced significant transformation (>3.0 times above controls) of 4 autologous (mean, 4.8), 6 homologous RA (mean, 14.3) and 6 nonRA cell donors (mean, 6.9). No significant transformation was induced by 1 RA-SF or by 8 nonRA-SF cultured with 5 autologous (mean, 1.3), 5 homologous nonRA (mean, 1.4) and 5 RA cell donors (mean, 1.2).

This study demonstrates that RA-SF, in contrast to nonRA-SF, can be mitogenic for human lymphocytes. The mitogenic response is a function of heat inactivated and appropriately diluted RA-SF and not of RA lymphocytes. Additional in vitro correlates of the reaction will be discussed.

Arthritis of Acute Sarcoidosis (AAS)

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There is paucity of information on synovial membrane and fluid changes in AAS. Therefore, we have studied 6 women and 4 men, aged 21 to 45, who fulfilled at least 3 of the following diagnostic
criteria: hilar adenopathy (8), arthritis (10), erythema nodosum (EN) (7), impaired delayed hypersensitivity (10) and noncaseating granulomata (NCG) on biopsy (9). Arthritis was the presenting symptom in 9, preceding or synchronous with EN, and lasted for 3 weeks to 7 months. Ankles and knees were by far the most commonly affected joints, with wrists, fingers, subtalars, elbows, shoulders and heels occasionally affected. Hypergammaglobulinemia was present in 7 patients, rheumatoid factor and hyperuricemia in 4 each and elevated ESR in 9. The hilar adenopathy resolved in 3 patients within 15 months, decreased in 1 and remained unchanged in 4. Eight patients are currently asymptomatic and on no medications.

Needle synovial biopsies were obtained in 6 patients and synovial fluid in 5. Despite marked clinical signs of inflammation in 4 patients, the fluids were in the noninflammatory range with lymphocyte and large mononuclear predominance. Light and electron microscopy of the synovial biopsies revealed mild, focal lining cell proliferation, minimal perivascular or scattered round cell infiltration, perivascular and subsynovial fibrosis, vascular occlusion and no NCG. The last finding may be due to the blind technic, or to actual absence of NCG.

In conclusion (1) a self-limited arthritis is very frequently the presenting symptom in acute sarcoid; (2) the synovial membrane and fluid in AAS show only mild changes; and (3) this might be related to the benign, short course of the arthritis.

Electron Microscopic Observation of Blast Transformation of Lymphoid Cells in Rheumatoid Arthritis

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Abundant lymphoid cells, which frequently distribute in a follicular pattern, are found in the rheumatoid synovial membrane. In this investigation, the ultrastructural characteristics and distribution of these cells have been studied in 10 synovial membranes obtained at synovectomy.

Lymphocytes and plasma cells were observed in dense accumulations around small blood vessels. The character of the cell populations differed in different accumulations. Some consisted almost entirely of pure populations of small lymphocytes; others consisted mainly of plasma cells. Still others showed transitional areas between groups of lymphocytes and groups of plasma cells. Frequently, migration of small lymphocytes from capillaries into almost pure populations of small lymphocytes was seen.

Blast cells in various stages of transformation were observed in the lymphoid collections. These had characteristic features—ie, increased amounts of cytoplasm with abundant RNP-granules in polyribosomal pattern and nuclei with diffuse chromatin pattern and large nucleoli. Occasionally, they had eccentrically located nuclei, concentrically arranged endoplasmic reticulum and well-developed Golgi areas, suggesting a transition from lymphocytes to plasmablasts and plasma cells. In areas around blast cells, injury to capillary walls and degeneration of fibroblasts and of the blast cells themselves were observed.

These findings suggest that in the rheumatoid inflammatory response, small lymphocytes derived from capillaries are transformed into immunoblasts, presumably as a result of antigenic stimulation. The stimulated cells may (1) produce local cytotoxic injury, either through direct cell to cell contact or by release of cytotoxic substances, and (2) undergo transition to plasmablasts and plasma cells which produce specific antibody. These phenomena appear to be of fundamental importance in relating the immune mechanism in rheumatoid arthritis to the local synovial inflammatory response and tissue injury.

Deforming, Nonerosive Arthritis of the Hands in Chronic Systemic Lupus Erythematosus (SLE)

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Review of a large group of SLE patients revealed 14 with ulnar deviation of the fingers without erosive changes demonstrable on careful radiologic study. The majority also had MCP joint subluxations. Many could correct these deformities spontaneously, as in Jacoud’s arthritis.

All have had 3 or more of the following: (1) skin lesions consistent with SLE, (2) alopecia, (3)
pleurisy, (4) pneumonic infiltrates, (5) pericarditis, and (6) glomerulonephritis. All had nonerosive involvement of other joints. All had positive LE preparations as well as positive fluorescent spot tests for antideoxyribonucleoprotein (anti-DNP), and 8 of the group for anti-DNA.

Disease duration was unusually long for SLE; 5 to 27 years from onset, average 14.5, median 12.5. Unexpectedly, 12 had abnormal Schirmer tests (<10mm), and at least 6 had definite keratoconjunctivitis by slit lamp. None had polymyositis or scleroderma. Less than 10% of our SLE controls had Schirmer tests <10mm.

The nonerosive arthritis with the described deformities and the prolonged mild course of the disease suggest that: (1) these cases may represent the chronic end of the SLE spectrum; or (2) they comprise a specific group of SLE with Sicca syndrome. SLE and Sjögren's syndrome are considered to be an uncommon, although not unknown combination. The probability of Sjögren's syndrome in this group of patients may be, perhaps, a reflection of the long duration of their disease.

The Articular Manifestations of Systemic Lupus Erythematosus (SLE): A Clinico-Pathologic Study

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Twenty-five patients with SLE and joint manifestations have been studied to correlate clinical and pathologic observations. All had positive LE preps and/or antinuclear factors, and multisystem disease including a characteristic skin rash, serositis, or renal disease. Patients with evidence of concomitant rheumatoid arthritis were excluded. Eighteen patients had frank arthritis. This was often symmetric and commonly preceded all other systemic manifestations. The arthritis was frequently evanescent and resolved within 24 hr without therapy. The knee, PIP and MCP joints were the most commonly involved. Three patients on steroid therapy developed aseptic necrosis of the hips (2), and the knee (1). The other patients had no joint deformities and x-rays showed no destructive changes. Four patients had only arthralgias. Synovial fluids had WBC's up to 4450 cells/cu mm, with cytoplasmic inclusions in many polys.

Synovial biopsies were obtained on 7 patients and studied by light and electron microscopy. All had some lining cell proliferation, and 5 had small amounts of superficial fibrin-like material. There were mild predominantly perivascular mononuclear infiltrates in all with diffuse inflammation, including many plasma cells in 2 and moderate numbers of neutrophils in 3. All biopsies showed microvascular obliteration with large endothelial cells, intraluminal inflammatory cells or platelet-fibrin thrombi. Some vessels showed gaps between endothelial cells. Clusters of tubular paramyxovirus-like inclusions were seen in the venular endothelium of one patient. No large electron dense deposits were seen in the vessel walls. Perivascular fibrosis and basement membrane reduplication was prominent. Nuclear and cytoplasmic debris was scattered throughout most synovial membranes, and deposits resembling degenerated nuclear material were seen in synovial phagocytes and in vascular endothelium. No nuclear debris was large enough to be considered a typical hematoxylin body. Biopsies on 2 patients with only arthralgias also showed most of the above changes including vascular obliteration and cell necrosis.

The articular manifestations of typical SLE appear to be mild and self-limited except for the occurrence of aseptic necrosis in 3 patients. Synovial findings are not diagnostic but suggest a prominent role for microvascular changes.

Detection of Red Cell Sensitization in Autoimmune Diseases by the Polybrene Technic

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Automated technics for the detection of red cell antibodies have provided extreme sensitivity, but have not been applied to autoimmune diseases. A modification of the Polybrene technic (Lalezari, P: Transfusion, 1968) was used to study red cell autoantibodies: red cells agglutinated by an antigen-antibody reaction were deaggregated by continuous exposure to heat at temperatures varying from 10 to 60° C. The recorded results provided characteristic curves, referred to as temperature gradient dissociation curves (TGDC). T 50% represented the temperature at which 50% of the
red cell aggregates dissociated. The indirect test determined serum antibodies; the direct test was a measure of cell-bound antibodies (in vivo sensitization). Optical density changes and T 50% corresponded to the quantity and the thermal characteristics of the antibody involved. In contrast to normal individuals and control patients, abnormal and characteristic TGDC and T 50% values were obtained in all patients who had various types of active autoimmune diseases. Included in this group were 15 patients with systemic lupus, 8 patients with idiopathic hemolytic anemia (IHA), 10 patients with hemolytic anemia secondary to lymphoma, and 10 patients with an autoimmune disease similar to LE but with negative LE preparations. The results may be summarized as follows:

(1) The direct Polybrene test in all cases studied was the most sensitive indication of autoimmunity, and was positive in all 14 cases of active LE studied. In 9 of these 14 patients, the direct Coombs test was negative or only a trace positive.

(2) T 50% (Direct test) in all 8 patients with IHA was >55° C. In contrast, in all patients with LE and LE-like syndromes, the T 50% was below 50° C. In lymphoma, both types of reactions were observed. In 3 patients T 50% was >60° C and in 7 below 45° C.

(3) In patients with IHA, therapy had no effect on the direct tests and their T 50% values. In contrast, in all secondary diseases, the T 50% was lowered or the test became negative after steroid therapy.

It is concluded that the Polybrene test is a diagnostic tool and can provide useful prognostic information in patients with autoimmune diseases.

**Inhibition of Collagen Synthesis in Chick Embryos by the Proline Analogue L-Azetidine-2-Carboxylic Acid**

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L-azetidine-2-carboxylic acid was previously shown (Biochim Biophys Acta 175:142, 1969) to be incorporated into the collagen synthesized by isolated cartilage from chick embryos. The collagen containing azetidine-2-carboxylic acid had a decreased content of hydroxyproline, hydroxylysine and glycosylated hydroxylysine, and it was not extruded from cells within 2 hr. In the present study, an attempt was made to use azetidine-2-carboxylic acid to inhibit selectively the synthesis of collagen in vivo. Azetidine-2-carboxylic acid in a dose of 500 μg/day was administered to chick embryos from Day 8 to Day 12. Control embryos showed a 5.4-fold increase in protein, and treated embryos showed a 3.6-fold increase in protein. The protein content of the treated embryos was therefore 67% of the control. The collagen content of the treated embryos, however, was only 28% of the control. Injection of *H-proline into the embryos on Day 11 or Day 12 indicated that the synthesis of collagen *H-hydroxyproline was inhibited about twice as much as the synthesis of other proteins. Collagen isolated from the embryos contained 4-10 residues per 1,000 residues of azetidine-2-carboxylic acid; but showed no significant differences from normal collagen in composition of other amino acids, melting temperature, shrinkage temperature, molecular size and formation of segment-long-spacing. These and previous observations in vitro indicate that azetidine-2-carboxylic acid produced a relatively specific inhibition of collagen synthesis in chick embryos by promoting the synthesis of a collagen which contained the analogue and which could not be extruded from cells at a normal rate. Since collagen contains more proline than most body proteins, and since prolyl residues play a critical role in determining the molecular conformation of collagen, it may be possible to develop proline analogues which will specifically inhibit collagen synthesis in conditions involving fibrosis of tissues.

**IgG Subclasses: Measurement by Radioimmunoassay in Normal and Hypogammaglobulinemic Sera**

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Clinical interest in the 4 subclasses of human γG globulin has been heightened by their observed biologic differences:—eg, in complement fixing activity, capacity to opsonize target cells for interaction with macrophages and catabolic rates. We have developed radioprecipitin inhibition assays (sensitivity 0.5 μg/ml) for quantification of the γG subclasses in serum or other fluids. Each assay employs specific monkey or rabbit antisera, *125*I-γG myeloma protein of a given subclass, and unlabeled...
myeloma globulin of that subclass as standard inhibitor. Values for normal human sera are seen in the table:

Sera of 9 patients with primary non-X-linked hypogammaglobulinemia (hypo-\(\gamma\)), without evidence of hypercatabolic loss, were tested to determine whether the low \(\gamma G\) levels involved symmetric or asymmetric depression of the 4 subclasses. Six sera displayed disproportionate percentages of subclasses, including very high \(\gamma G3\) and low \(\gamma G1\) and/or \(\gamma G2\) (3 cases) and less marked imbalances in 3 other cases involving high \(\gamma G4\) or high \(\gamma G2\) or low \(\gamma G2\). Although half-lives for each subclass have not yet been measured in these patients, the subclass imbalances cannot be readily explained by differential catabolic rates because of the variability in the observed patterns. Therefore, the data are tentatively interpreted to mean that the block in \(\gamma G\) synthesis in hypo-\(\gamma\) often does not affect all subclasses equally.

| Total \(\gamma G\) | \(\gamma G1\) | \(\gamma G2\) | \(\gamma G3\) | \(\gamma G4\) |
|------------------|-----------|-----------|-----------|-----------|
| Mean (mg/ml)     | 8.8       | 6.7 (64%) | 2.9 (28%) | 0.54 (5%) | 0.36 (3%) |
| Range (mg/ml)    | 7.1–10.2  | 5.3–7.6   | 1.9–4.1   | 0.23–0.78 | 0.12–0.72 |

**Skin Blood Flow in Scleroderma (Systemic Sclerosis) and Raynaud’s Syndrome**

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Skin blood flow of the dorsal finger has been compared in 14 normal subjects, 6 patients with Raynaud’s syndrome without scleroderma, and patients with varying degrees of scleroderma (29 studies in 21 patients). Each subject received \(^{133}\)Xe intracutaneously after 1 hr in a cool room (17° C), and after subsequent rewarming of the opposite arm in a 44° C waterbath; body, skin and air temperatures were monitored. Cutaneous clearance was expressed as half-times (\(t_{1/2}\)) of isotope washout curves which, when biphasic, were resolved graphically. After cooling, \(^{133}\)Xe was cleared with a \(t_{1/2}\) of 2.5 min in normal subjects (14), a \(t_{1/2}\) of 4.8 min in patients with Raynaud’s syndrome (6), and a \(t_{1/2}\) of 43.3 min in patients with diffuse scleroderma (24 studies in 21 patients). Individual clearances in the patients with scleroderma suggested heterogeneity not apparent clinically: 6 subjects with near normal clearance (\(t_{1/2} = 3.3\) min) contrast with 18 studies in 15 patients whose blood flow-dependent clearance was essentially obliterated (\(t_{1/2} = 56.6\) min). Four patients separated clinically by extensive central scleroderma, no Raynaud’s phenomenon, and sparing of hand and finger skin, had normal clearance (\(t_{1/2} = 2.2\) min). After 4–6 weeks of oral guanethidine therapy (30–50 mg/day), clearance while cool reverted to normal levels in 3 patients with scleroderma (\(t_{1/2}\) before = 67.7 min, after = 2.8 min) and did not change in 2 similar patients (\(t_{1/2}\) before = 56.8 min, after = 57.8 min).

Clearance after warming was similar in normal subjects (14, \(t_{1/2} = 1.7\) min), scleroderma patients (24 studies, 21 patients, \(t_{1/2} = 1.8\) min) and patients with Raynaud’s syndrome (6, \(t_{1/2} = 1.9\) min), although rewarming in the scleroderma group was delayed and incomplete. Guanethidine-treated scleroderma patients demonstrated a prolonged warm skin clearance (5, \(t_{1/2}\) before = 2.1 min after = 3.3 min) associated with a diminished warming response. Thus, a cold-induced interruption of the microcirculation of the skin has been demonstrated in scleroderma patients and, to a lesser degree, subjects with Raynaud’s syndrome. This defect may be relevant to the pathogenesis of scleroderma and would appear to be more approachable therapeutically than the irreversible ‘hide-bound’ fibrosis, since guanethidine in moderate doses blocked the response to cold in 3 of 5 patients treated.

**Abnormal Navicular-Lunate Separation in Rheumatoid Arthritis**

Martin D. Lidsky, Lois Collins, June Morland and John T. Sharp, Houston, Texas

An increase in joint space is reported to be a rare radiologic finding in rheumatoid arthritis. During a review of serial roentgenograms of the wrist from patients with definite or classic rheumatoid arthritis, abnormal separation of the navicular and lunate was observed with unexpected frequency, namely in 19 of 110 patients (17.3%). In patients whose earlier films did not show the
widened interosseous space, the separation was observed from 29 to 325 months after onset of clinical disease. In 6 patients, the separation was seen on the first available film obtained from 35 to 132 months after onset of disease.

Subcutaneous nodules in the elbow region, present in 13 of 19 patients with abnormal navicular-lunate separation, were not significantly more frequent than in patients without the separation. Sera from 16 of the 19 patients with separation were assayed for rheumatoid factor activity, and all 16 sera were positive. Evaluation of erosions and joint space narrowing in the fingers and wrists using a previously reported semi-quantitative method disclosed no difference between patients with and without abnormal navicular-lunate separation.

In conclusion, the study revealed that widening of the space between the navicular and lunate is a common finding in rheumatoid arthritis. The patients with this finding displayed no other distinguishing characteristic.

Spondylodiscitis: An Unusual Feature of Ankylosing Spondylitis
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Spondylodiscitis is a lesion in ankylosing spondylitis commonly misinterpreted as infection or fracture. The radiologic appearance of spondylodiscitis is strikingly characteristic, with erosion of vertebral bodies adjacent to a disc and marked sclerosis of the surrounding bone.

Eleven male patients, who have had spondylodiscitis, have been followed for periods ranging from 6 weeks to 27 years. The spondylodiscitis followed the onset of ankylosing spondylitis as early as 2 years and as late as 30 years. Five patients had pain at the site which led to the diagnosis. Six patients developed spondylodiscitis insidiously without any apparent increase in their symptoms.

The ankylosing spondylitis was complicated by peripheral joint involvement in 5 patients and by iritis in 2. Two patients had fracture dislocation of the spine with transient paraplegia or quadriplegia. The fracture healed spontaneously in 1 and required surgical fusion in the other. The spondylodiscitis developed 2 and 22 years later at different levels. One patient had arytenoid fixation with stridor requiring tracheotomy. None had aortitis.

In these patients, spondylodiscitis has been a benign lesion, often asymptomatic and without serious complications in an average follow-up of 15 years. This natural history should be taken into consideration before recommending surgical treatment or prolonged immobilization.

Antirheumatic Action of Gold Salts Observed in Chrysotherapy
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Several hypotheses have been proposed for the antirheumatic action exerted by gold salts. These, however, are largely based on theoretic considerations and laboratory studies rather than on conditions existing in patients receiving chrysotherapy. We have conducted serial serum gold analyses in 20 patients receiving chrysotherapy. Maximum serum values were observed after 20 consecutive weekly injections (50 mg each) of gold thiomalate.

| Hours after injection | 2  | 24  | 48  | 72  | 96  | 120 | 144  | 168 |
|----------------------|----|-----|-----|-----|-----|-----|------|-----|
| Mean serum gold level (µg%) | 544| 484| 427 | 355 | 347 | 306 | 276  | 255 |

The highest serum gold values were observed 2 hr after injection (mean 545 µg%) followed by progressive decline until the next injection. Synovial fluid when available for examination approached 50% of serum values. This concentration of gold, when added to cell-free synovial fluids obtained from patients with rheumatoid arthritis (RA) not on chrysotherapy, was inhibitory to acid hydrolases (acid phosphatase, β-glucuronidase). Suppression of intracellular granulocyte acid hydrolase activity was not observed when cells were incubated in plasma containing gold at 600 µg%. However, leukocyte acid hydrolase suppression has been reported at higher gold levels (200 times) than those achieved in clinical situations. Moreover, the cellular acid hydrolase activity in RA patients receiving
chrysotherapy did not differ significantly from those RA patients who were not receiving the medication. These observations suggest that gold acts by acid hydrolase suppression in extracellular fluid.

**A Study of Polymyositis (PMS)**

**ISRAEL MACHTEY,** Petah Tiqva, Israel

Different aspects of PMS in 19 patients (4 men, 15 women; average age 47 years) were analyzed. In 5 cases PMS was unassociated with other diseases. In 3 patients rheumatoid arthritis (RA) was diagnosed: in one, PMS preceded the onset of RA. Other associated diseases were: Sjögren’s syndrome, regional ileitis, hypersensitivity reaction, recurrent gonarthritis, myotonia and psoriasis. Malignant growths were found in 5 cases (26%); in 4, PMS preceded the diagnosis of malignancy.

Clinically, every patient showed a definite myositis involving the proximal muscles of the extremities. Arthralgia or arthritis was present in 12 patients, and 5 patients had alimentary tract involvement. All patients were febrile and some had very high temperatures.

Laboratory findings included: a marked creatinuria, an accelerated sedimentation rate and, in some cases, a positive latex reaction or a positive LE cell preparation. A significant hypoalbuminemia (under 3.0 g/100 ml) was found in 10 patients (53%). The electromyogram was abnormal in all but 1 case. Elevated serum enzyme activity (transaminase, creatine, kinase or lactic dehydrogenase) was found in 7 patients only; this low figure may be due to a lack of sufficient estimations in many cases. Skin or muscle biopsies were obtained from 7 patients; all showed pathologic changes, though per se not always specific for PMS. Four patients died; in 3 cases an autopsy was performed. The chief findings in 1 of these cases were regional ileitis and ulcerative colitis; in the second, a necrotizing glomerulitis; and in the third, a ruptured intracranial aneurysma. Illustrative cases are discussed briefly.

**Prolonged Alkylating Drug Therapy is Beneficial in Systemic Scleroderma (PSS)**

**ALLEN H. MACKENZIE,** Cleveland, Ohio

No major improvement in PSS patients with arrest of progressive features has been ascribed to existing therapies. Preliminary studies using Chlorambucil (LK) in treating PSS indicate that major improvement with arrest of progression does occur and can be objectively documented. In 11 PSS patients (6F, 5M), the diagnoses were based upon sclerotic skin edema, Raynaud’s phenomenon, fingertip ulceration, telangiectases, hyperpigmentation, measured limitation of finger flexion, positive skin biopsy, basilar pulmonary infiltrates, reduced pulmonary function tests (PFT) (including CO diffusion and ventilation studies) and exertional dyspnea. Added findings were PSS heart disease, polymyositis, dysphagia, esophageal aperistalsis, small bowel PSS, phalangeal tuft resorption, calcinosis, restricted mouth gape, synovitis, increased globulins and sedimentation rate. Range of hand motion was measured in millimeters during maximum empty grasp from fingernail to palmar flexion crease. Gape was measured between medial incisors. Skin pliability and Raynaud’s were hard to quantify. All 11 patients had systemic disease progressing rapidly in 4, moderately in 5 and slowly in 2. No patients with benign acrosclerosis, nonprogressive PSS, or benign CRST syndrome were included. LK, 0.1 mg/kg/day, was administered orally, mean duration 18.3 months (range 9–34), totaling 17 man-years of exposure. Results: progressive PSS involvement of every organ system was halted in the 11 patients studied. Measurable objective response to therapy required 6–12 months; none died. All fingertip ulcers healed; no new ones formed. Fingertip to palm measurement decreased significantly (>5 cm); gape increased (mean 10 mm); body weight increased (mean 14 lb); PFT improved significantly (>20% of predicted normal) in 55% and declined in none. Dyspnea was reduced. Skin sclerosis decreased greatly if edematous. “Dry” collagen did not change. Conclusions: Chlorambucil therapy appears to halt progression of the systemic and cutaneous features of PSS based on objective measurement of range of motion, PFT, x-rays, state of subjective well-being and regained functional capacities. No treatment complications were identified. PSS arrest in all 11 patients under treatment during a 1–3 year span is most anomalous; 7 should have showed progression and 2 should have died. I recommend a blind controlled study without crossover in a larger patient population.
Indomethacin in Reiter's Syndrome

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Among the more recent drugs used in the treatment of rheumatic diseases, indomethacin occupies an important place. The use of this drug in Reiter's syndrome, while generally effective, has up to now been limited to few patients. During the past 5 years, we used indomethacin in treating 22 consecutive patients with Reiter's syndrome, all of them exhibiting the classic triad (urethritis, conjunctivitis, arthritis).

Of the 22 patients, all men, the mean age was 31 years, the range 16 to 53. In addition to the symptom triad, keratodermia was present in 10, balanitis in 9 and oral lesions in 6.

The response to indomethacin was good in 18 patients, fair in 2 and poor in 2. No specific features differentiated the responders from the non-responders. Suppression of arthritis occurred on an average of 4 days (from 2 days to 2 weeks). As expected, the antirheumatic drug had no effect on the nonarticular features of the disease.

The duration of required indomethacin therapy was variable, lasting from 2 months to as long as 1 year. Seven patients had a recurrence, each responding as he had during his initial attack: 5 good, 1 fair, 1 poor. The average dose of indomethacin was 150 mg (from 75 to 200 mg). Side effects included transient headaches in 6 patients and gastrointestinal upset in 4. In no instance was it necessary to discontinue the drug because of adverse reactions. From this experience, we conclude that indomethacin may be considered a useful antirheumatic drug in the management of patients with Reiter's syndrome.

The Glycosaminoglycans of the Articular Cartilage from Aged, Normal and Osteoarthritic Human Hip Joints

Henry J. Mankin and Louis Lippiello, New York, New York

The cartilages from the hip joints of 13 normal and 15 osteoarthritic humans were analyzed for glycosaminoglycan (GAG) content and distribution. The GAG's were separated by elution with CPC on a short cellulose column by the technic of Svejcar and Robertson after digestion of the tissue with pronase and papain. The eluates were identified by a variety of methods, including determination of molar ratios, n-acetyl-hexosamine determinations after hyaluronidase treatment, and thin layer chromatography of unhydrolyzed and hydrolyzed GAGs.

From the data obtained, it was demonstrated that cartilage from arthritic patients showed a significant increase in the concentration of chondroitin-4-sulfate and a significant decrease in keratan sulfate, with only slight changes in the total amount of GAG present. Calculations of the molar ratios showed variation in sulfation with both chondroitin-4 and -6 sulfate appearing in the "super-sulfated" state in the arthritic cartilage.

The data lead to speculation regarding the process of osteoarthritis, and it is concluded that the changes seen are more likely to represent an altered pattern of synthesis rather than selective degradation. Since the changes suggest a younger cartilage, a theory is advanced that the chondrocyte responds to the chronic stress of osteoarthritis by modulation to a chondroblastic phase.

Observations on the Value of Traction During Roentgenography of the Hip

William Martel, Andrew K. Poznanski and William S. Smith, Ann Arbor, Michigan

Traction on the hip during roentgenography often produces subluxation with intra-articular gas due to the "vacuum phenomenon". This pneumoarthrogram clearly portrays the joint space and articular cartilage. When traction causes widening of the interosseous space without intra-articular gas, it indicates excessive joint fluid. These postulates and the clinical usefulness of this technic were evaluated in 75 hips, 51 of which were normal controls. An arthrogram occurred in 45 of the normal hips and in one-third of the abnormal ones. Intra-articular fluid was verified in 3 cases in which distraction occurred without the vacuum phenomenon. Postmortem experiments indicated that this is strictly a physical phenomenon and that in newborn infants as little as 1 ml of injected fluid is sufficient to preclude the vacuum effect. In none of the normals did traction cause widening of the interosseous...
distance without intra-articular gas. The characteristic "radiolucent crescent line" in osteonecrosis appears to be due to the "vacuum phenomenon" within the necrotic bone.

The method’s simplicity and apparent reliability make it particularly useful in the hip where effusion is difficult to diagnose. It is an atraumatic and potentially valuable technic for radiologic investigations of various hip diseases for it makes evaluation of the articular cartilage possible.

**Inhibition of Human Erythrocyte Pyrophosphatase Activity by Calcium, Cupric and Ferrous Ions**

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The deposition of calcium pyrophosphate crystals in articular cartilage (pseudogout syndrome) is frequently associated with hyperparathyroidism (8%). Numerous cases with coincident hemochromatosis and a few cases with associated Wilson's disease have been reported.

A sensitive assay was developed to measure the rate of hydrolysis of $^{32}$P-O-P to $^{32}$Pi (which was then precipitated selectively) and applied to the study of the soluble pyrophosphatase (PPiase) in RBC hemolysates. Purity of $^{32}$P-O-P was ascertained by thin layer chromatography. Final concentrations were PPi 1.5 mM (100 x $K_m$), Mg$^{++}$ 1.75 mM, tris Cl 33.3 mM, pH 7.7. PPiase activity in 13 randomly selected patients and normal controls averaged 0.026 μM/min/mg protein (range 0.021 to 0.028).

Fe$^{++}$, Fe$^{+++}$, Cu$^{++}$ and Ca$^{++}$ did not act as cofactors when substituted for Mg$^{++}$, and all but Fe$^{++}$ markedly suppressed PPiase activity (> 95%) even in the presence of excess Mg$^{++}$.

The ion products of Ca$^{++}$ and the elevated synovial fluid PPi in pseudogout reported by Fleisch et al are but one tenth the values that we have found necessary for in vitro precipitation of calcium pyrophosphate, so that even in hyperparathyroidism factors other than a simple increase in ion product must be sought. If inhibitory concentrations of the divalent cations obtain in the affected tissues in the above mentioned associated diseases, and if PPiases vital to Ppi homeostasis are inhibited by them, then these observations provide a working hypothesis to explain the association.

**Factors Affecting Survivorship in Polymyositis**

Thomas A. Medsger, Jr, Harry Robinson and Alfonse T. Mas, Memphis, Tennessee

No life-table analysis of factors affecting survivorship in polymyositis has been reported. From 1947 through 1968, 124 cases of documented polymyositis, including 56 patients with dermatomyositis, were identified; these patients were described previously in an epidemiologic study. The group consisted of 74 females and 50 males; 86 were treated with corticosteroids and only 5 had associated malignancy. Follow-up was obtained on all but 6 individuals, and the data were analyzed by actuarial life-table methods to determine the significance of various factors on survivorship.

Forty-two patient deaths occurred with an average interval of 1.5 years after diagnosis. The 82 survivors were observed an average of 4.8 years after diagnosis. Cumulative life-table survivorship percentages (Table) showed the greatest mortality in the first year, particularly in the first 3 months. At the time of first diagnosis, 14 patients had non-malignant pulmonary infiltrates on chest x-ray; 11 (79%) of these persons died, whereas 31 (28%) of

| Patient category          | No. of patients | No. of dead | $\frac{1}{4}$ | 1   | 3   | 5   | 7   |
|--------------------------|----------------|-------------|---------------|-----|-----|-----|-----|
| Pneumonitis              | 14             | 11          | 50            | 43  | 36  | 36  | 12  |
| No pneumonitis: Adult Negro | 35          | 17          | 79            | 67  | 54  | 41  | 31  |
| Adult White              | 51             | 12          | 96            | 87  | 79  | 75  | 59  |
| Children                 | 24             | 2           | 100           | 95  | 89  | 89  | 89  |
| All patients             | 124            | 42          | 87            | 77  | 69  | 65  | 52  |

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the remaining 110 cases without "pneumonitis" died ($p < 0.001$). Further analysis of patients without pulmonary infiltrates revealed that children had a better survivorship than adults ($p < 0.05$), and that adult whites had a better survivorship than adult Negros ($p < 0.05$). Adult Negros with greater muscle weakness at first diagnosis had a higher mortality than those with lesser weakness ($p < 0.05$). Excluding the patients with malignancy did not alter these conclusions. Corticosteroid therapy was not found to improve long-term survivorship in this series.

**Inhibition of Phagocytosis and Killing of Bacteria by Gold In Vitro**  
**RONALD P. MESSNER, ERIK E. CARLSON and JOAN G. JELINEK, Albuquerque, New Mexico**

Gold salts are capable of inhibiting the action of certain lysosomal enzymes. Since intracellular killing of bacteria after phagocytosis is mediated by lysosomal enzymes, the present work was undertaken to determine if gold salts were capable of altering the ability of human neutrophils to phagocytize and kill common pathogenic bacteria.

Serial dilutions of gold sodium thiomalate (a salt which contains 50% gold) were added to an in vitro phagocytic system containing bacteria and normal human leukocytes in a 1:1 ratio and fresh normal human serum as opsonin. Killing of bacteria was measured by quantitative pour plate counts at intervals during a 120 min incubation. Inhibition of phagocytosis and killing of proteus species was noted with concentrations of gold sodium thiomalate as low as 200 µg/ml. Killing of *Pseudomonas aeruginosa* progressively decreased as the concentration of gold sodium thiomalate was increased from 400 µg/ml to 1600 µg/ml; however, no significant decrease was noted with concentrations below 400 µg/ml. Killing of *Staphylococcus aureus*, *Klebsiella pneumoniae* and group D *streptococcus* was inhibited by concentrations ranging from 1000 to 1200 µg/ml; while the killing of *Escherichia coli* was unaffected by concentrations up to 1600 µg/ml.

The concentration of gold required to inhibit the bactericidal activity of neutrophils is 30 to 400 times that attained in serum with conventional weekly intramuscular chrysotherapy. It is possible, however, that accumulation of gold in areas of inflammation might result in concentrations sufficient to impair the bactericidal activity of neutrophils in vivo. The sixfold difference in concentration required to inhibit killing of *S. aureus* compared to proteus species and the inability to inhibit killing of *E. coli* by neutrophils suggests that gold selectively inactivates the mechanisms responsible for the bactericidal action of the human neutrophil.

**Rat Mycoplasma Arthritis: An Electron Microscopic Study**  
**DAVID MILLS and JOHN WARD, Denver, Colorado and Salt Lake City, Utah**

*Mycoplasma arthritidis* (MA) infection in the rat has usually been thought to result in an acute suppurative arthritis. This study shows the transition into a chronic disease and the presence of the organisms in the earliest stage.

MA was given by IV or intra-articular injection to adult rats. With a dissecting microscope, knee synovium was removed and processed for electron microscopy by routine methods. At 3 hr after joint injection, MA was seen attached to lining cell surfaces. By 8 hr after either route of injection, polymorphonuclear (PMN) infiltrate was present in the lining cell layer and contained inclusions which were probably MA. By 24 hr PMN's thickly populated the lining layer with some degeneration of both lining cells and PMN's. Between 1 and 3 days PMN's completely replaced the lining layer; and material which was neither typical fibrin nor typical collagen interlaced the cells. Small vessels near the surface were occluded. At 12 days large macrophages were now seen and contained partly digested PMN's (similar to Reiter cells). By 20 days, the lining layer was reformed with synoviocytes of increased number and size, as well as giant cells and rare mitotic figures. Plasma cells were seen in perivascular areas. PMN's were fewer and were frequently degenerating.

In conclusion, the arthritis following MA initially destroys the lining layer and is replaced with hypertrophic and hyperplastic cells. Plasma cells and giant cells appear as the PMN infiltrate regresses. There are many histologic similarities of MA synovitis and chronic rheumatoid arthritis.
Chronic Salicylate Induced Gastrointestinal Bleeding: Quantitative Response to Therapy

GREGORIO MINTZ, ANTONIO FRAGA, LUIS CERVANTES and ALFREDO CUARÓN, Mexico City, Mexico

Occult gastrointestinal bleeding is a known side effect of chronic acetylsalicylic acid (ASA) therapy, and its management has not received too much attention. Since the acute bleeding episodes secondary to ASA administration respond well to the usual acute peptic ulcer management, the present study was undertaken to assess the influence of a modified peptic ulcer regimen on the amount of the chronic daily blood loss of these patients.

The 30 subjects were divided into 3 groups of 10: (1) normal controls, (2) rheumatoid arthritis taking only 3 gm of ASA per day, (3) rheumatoid arthritis taking ASA plus 9/10 oxyphenylbutazone, 4/10 hydroxychloroquine and 1/10 prednisone. The patient's red cells were labeled with Cr	extsuperscript{51} and injected intravenously, and 24-hr stool specimens on 3 consecutive days were measured for blood loss.

The mean amount of blood loss in the controls was 0.86 ± 0.14 ml/day. In group 2 it was 2.55 ± 0.54 ml/day and in group 3 blood loss was 2.64 ± 0.9 ml/day. Compared with the control group the results of groups 2 and 3 have a p = 0.01. The 7 patients who bled in excess were given a bland diet, anticholinergic drugs and antacids for 15 days. A decrease of blood in the stools was found from a previous mean of 5.13 ± 0.85 ml/day to 2.5 ± 0.43 ml/day (p = 0.005).

In all subjects upper and lower GI pathology was ruled out prior to the study, therefore our results suggest that chronic blood loss in these patients was due to minute mucosal bleeding that can be reduced, although not to normal levels, by the administration of a modified peptic ulcer regime. Simultaneous administration of ASA and other anti-inflammatory drugs did not significantly modify chronic blood loss and its response to ulcer management.

Lymphocyte Cytotoxic Antibodies in Systemic Lupus Erythematosus and Other Connective Tissue Diseases

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We recently showed that sera from patients with systemic lupus erythematosus (SLE) often contain lymphocytotoxic antibodies. To determine the prevalence of these antibodies in connective tissue diseases, 97 coded sera from patients with SLE, rheumatoid arthritis, rheumatic fever, polymyositis, scleroderma and polyarteritis were tested by a microassay technic for antibodies cytotoxic to lymphocytes from 38 healthy, unrelated persons. Twenty-seven of 31 patients with SLE (87%) had lymphocytotoxic antibodies in their circulation on one or more occasions. Of the 43 lymphocytotoxic sera from these 27 patients, 40 were obtained when the disease was active and 3 sera were from 2 patients in remission. Forty of 46 sera (87%) from patients with active disease contained cytotoxic antibodies. Only 3 of 7 sera (43%) taken during remission were positive. The 3 patients in whom SLE was suspected on a clinical basis all had circulating lymphocytotoxic antibodies. Cytotoxic antibodies were also found in sera from 3 of 6 patients with periarteritis, 2 of 6 patients with scleroderma, and 2 of 14 patients (14%) with rheumatoid arthritis. No antibodies were found in sera of 7 patients with discoid lupus, 6 patients with polymyositis or 6 patients with rheumatic fever. Since lymphocytotoxic antibodies occur infrequently in the general population, their presence in SLE, scleroderma and periarteritis is unique. The significance of these antibodies in the pathogenesis of the disease remains to be determined.

Personality, Disease Parameters and Medication in Rheumatoid Arthritis

HARVEY MOLDOFFSKY and ARTHUR I. ROTHMAN, Toronto, Canada

This study examined the inter-relationships between patients' personalities as defined by the Cat-
defined by medication. The sample consisted of 56 rheumatoid patients consecutively admitted to a Rheumatic Diseases Unit.

In order to compare patient groups for each disease parameter and drug family in terms of comprehensive characterization of personality, in these two phases of the study, a multivariate data analysis was used. Chi-square tests of association were used to determine whether relationships existed between the disease parameters and drug usage.

The personality profile of the total sample revealed tendencies to low ego strength, anxiety and dependency—characteristics that are not unique to rheumatoid patients.

No overall relationship existed between patients' personalities and any of the indices of disease activity. However, a significant relationship was found between patients' personalities and the dependency upon oral corticosteroid drugs. In comparison to those who had never received steroids, those who had been receiving this drug, were found to be characteristically more depressed and taciturn, complaintive and demanding, and dependent and easily upset. Yet, excluding ASA which was routinely administered to all patients, no significant relationships existed between any drugs given and disease parameters, except in the case of treatment with gold salts. Here the administration of gold salts was associated with both a moderately high titer of the latex fixation test (p < 0.05) and a progressive or persistent course of illness (p < 0.02).

### The Effect of Iron-Dextran on Experimental Synovitis in the Guinea Pig

**Alastair G. Mowat, Thomas F. Disney and John H. Vaughan, Rochester, New York**

Disturbance of iron metabolism is a prominent feature of rheumatoid arthritis, and large quantities of iron have been found in the synovial tissue in this disease. The mechanisms by which the iron reaches the synovial tissue and their actions, if any, in this site are unknown.

An acute, self-limiting synovitis has been induced in guinea pig elbow joints by a single intra-articular injection of Nystatin (10,000 IU). This synovitis has been utilized to study the effects of intra-articular injections of iron-dextran (100 and 200 μg), gold thiosulphate (200 and 400 μg) and hydrocortisone acetate (250 μg). The synovitis has been assessed clinically and by an external counting method using radioactive iodinated human serum albumin (RISA) as a vascular marker.

Significant (p < 0.005) control of the synovitis has been achieved using all 3 drugs. Iron has been shown to be twice as effective as gold on the basis of elemental weight. Dextran alone did not alter the synovitis. Further studies have demonstrated significant control (p < 0.005) of the synovitis by the injection of iron-dextran (2.5-5.0 mg) into the hind limb muscles.

The results confirm the anti-inflammatory effects of gold and hydrocortisone and demonstrate similar properties for iron-dextran. Iron may be selectively taken up from the joint fluid or plasma by inflamed synovial tissue. This mechanism could explain the high concentration of iron in the synovial tissue and the low plasma iron in patients with rheumatoid arthritis.

### Chemotaxis in Rheumatoid Arthritis and Other Connective Tissue Diseases

**Alastair Mowat, Oxford, England and John Baum, Rochester, New York**

Infection is a recognized feature of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), but the reasons for its increased frequency are unknown. As an initial study of the function of the polymorphonuclear leukocyte (PMN), a modification of the Boyden technic using peripheral blood leukocytes was developed. A standard chemotactic source (casein and human complement) was used throughout the study. Our modified method is simple and reproducible using small (≤ 10 ml) volumes of peripheral blood. We have studied 24 patients with RA and compared them with age and sex-matched normal controls. Chemotactic values for normals were 555 ± 52 and for RA 320 ± 72 (p < 0.0005). Further analysis of the results showed no relationship to clinical activity of the disease, to drugs used in treatment or to latex titer.

Possible reasons for this abnormality are: decreased activity due to prior ingestion of macromolecules, a primary metabolic deficiency of the PMN, or interference by a globulin coating on the cell. A model for the former mechanism has been demonstrated by the prior incubation of normal PMN's with varying concentrations of iron-dextran.
aggregates. Studies on the latter by immunofluorescent methods are in progress. Since studies using the PMN's from 31 diabetic patients (414 ± 96) have shown restoration to normal values after incubation with insulin in those tested, a model for a metabolic deficiency of the PMN has also been demonstrated. A further finding in 3 patients with severe connective tissue diseases (RA, SLE and polymyositis) was that initially abnormal values (392, 296, 208) returned to normal (629, 596, 497) after a few days' treatment with large doses of steroids.

We have thus demonstrated a deficiency of the peripheral blood polymorphonuclear leukocyte as a possible reason for increased infection in patients with connective tissue diseases.

Skeletal Status in Rheumatoid Arthritis

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Lack of objective technics has made it difficult to determine the extent of osteoporosis in rheumatoid disease. The University of Wisconsin Bone Mineral Laboratory has developed two quantitative in vivo methods of skeletal status measurement: bone mineral content by monoenergetic photon absorption, and bone stiffness by vibrational analysis. The bone mineral content (BM) and width (W) of the midshaft and distal end of the left radius were measured in 26 women with ARA classic or definite rheumatoid arthritis and age-matched nonarthritic controls. Six rheumatoid subjects had received corticosteroids (RA-S); 20 had never received steroids (RA). BM/W at the midshaft or the distal end of the radius in the RA patients did not differ significantly from the values of their controls. The RA-S patients differed significantly from their controls. The average bone mass at the midshaft was 0.51 g/sq cm compared to 0.75 g/sq cm for the controls, a 32% difference (p < 0.03). At the distal end of the radius, steroid-treated patients had an average of 0.34 g/sq cm compared to 0.55 g/sq cm for their controls, a difference of 38% (p < 0.03). The product of ulnar resonant frequency times length (FL) was determined as a measure of bone stiffness in rheumatoid women and their controls. Sixteen RA patients had FL values 20% lower than their controls (p < 0.03). Seven RA-S patients had FL values 38% lower than their controls (p < 0.01). Thus, both bone mineral and stiffness are low in steroid treated rheumatoids. In nonsteroid treated rheumatoids bone stiffness is low despite apparent normal bone mineral content.

Inhibition of Lupus Erythematosus Cell Phenomenon in Uremia

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Immunologic reactions may be abnormal in patients with uremia. We present data indicating that the LE cell phenomenon may be suppressed in such patients. LE cell tests were negative in 2 patients prior to peritoneal dialysis, whereas tests after dialysis were positive. The first patient, a 21-year-old male, experienced rapid progression of renal insufficiency, thought to be due to subacute glomerulonephritis. Peritoneal dialysis reduced the BUN from 150 to 64 mg%, and LE cell preparations became strongly positive. Immunohistologic findings on renal biopsy and at autopsy were classic for systemic lupus erythematosus (SLE). The second patient, a 36-year-old female, was known to have SLE with progressive renal insufficiency. With peritoneal dialysis the BUN fell from 219 mg%, (at which level LE cell tests were negative) to 68 mg%, and a positive LE cell preparation was obtained.

The mechanism of inhibition has been studied with tests designed to distinguish sensitization and phagocytosis phases of the LE cell phenomenon. Mouse liver nuclei exposed to SLE serum diluted with normal serum form "loose bodies" which can be stained and evaluated (stage one) or resuspended in normal serum and presented to leukocyte preparations for observation of LE cell formation (phase two). Substitution of uremic serum in the first phase results in inhibition of sensitization. However, uremic serum has no effect on the second phase. Studies with urea as inhibitor have given inconsistent results. They are currently being pursued with fluorescent antibody technics.

Conclusions: (1) The LE cell phenomenon may be suppressed in patients with uremia; (2) This suppression has been diagnostically confusing; (3) Inhibition is relieved by peritoneal dialysis; (4) Inhibition is attributable to a dialyzable serum factor; (5) Inhibition affects the primary reaction of nucleoprotein with LE globulin.
Necrotizing Angiitis with Drug Abuse

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A necrotizing angiitis indistinguishable from periarteritis nodosa has been observed and studied in 10 young drug addicts. The 4 females and 6 males, ages 17-31 years, had used the entire spectrum of narcotics, depressants, stimulants and hallucinogens. Methamphetamine alone or in combination with d-lysergic acid diethylamide or with heroin was used commonly.

The clinical presentation varied from complete lack of symptoms in 2 patients to pleomorphic signs and symptoms including hypertension, abdominal pains, arthralgias and myalgias, renal failure, pulmonary edema and peripheral neuritis.

The vascular changes of periarteritis nodosa, muscular artery aneurysms and sacculations were identified on selective visceral and renal angiograms. There was a predilection for vascular bifurcation sites and the hilar regions of the abdominal viscera. Lesions were identified in the kidney, liver, pancreas and the small bowel. Confirmation of the angiographic findings was noted in 4 post-mortem studies where generalized vascular changes including healed and chronic lesions were identified.

The etiologic agent of this form of necrotizing angiitis is unclear because of the multiplicity of injected substances and the high probability of contamination of "street" and homemade drugs. Methamphetamine appears to be the common denominator.

A New Rheumatic Syndrome Associated with Reticulohistiocytic Granuloma

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A syndrome of dermatomyositis, polyarthritis and reticulohistiocytic granuloma is reported. A 42-year-old white male developed bilateral symmetric polyarthritis of the hands, knees and shoulders at age 40. This was followed by the development of a maculopapular erythematous rash on the arms, heliotrope discoloration of the eyelids, muscle atrophy and weakness, dysphagia and respiratory muscle insufficiency. Electromyogram and serum enzymes were abnormal, but muscle biopsy was normal. Based on a presumptive diagnosis of dermatomyositis, he was treated with systemic corticosteroids and showed definite but not permanent improvement. Two years after the onset, his arthritis worsened and many yellow to orange papules, 0.5-1.5 cm in diameter, developed over the finger, face and scalp. A biopsy of one of these demonstrated the changes of reticulohistiocytic granuloma; a synovial biopsy showed the same histologic pattern.

Previously, reported cases of reticulohistiocytic granuloma have notably been associated with abnormalities of lipid metabolism, thyroid dysfunction and rheumatoid arthritis. The following studies were performed to determine how this syndrome is related to those previously reported: serum cholesterol and triglyceride determinations, serum lipid electrophoresis, thyroid function studies, quantitative immuneelectrophoresis, latex fixation titers and synovial analysis. This is the first known case of dermatomyositis in association with reticulohistiocytic granuloma.

Relation of the Carpel Tunnel Syndrome (CTS) to Activity of Acromegaly

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Median nerve compression in acromegaly is caused by increased endoneurial and perineural connective tissue as well as an edematous synovial tissue reaction of flexor tendons. Two previously untested but related hypotheses were tested: (1) that CTS indicates "active" acromegaly and (2) that effective treatment of acromegaly relieves CTS.

Records of 100 new patients with classic acromegaly seen between Jan 1962 and June 1968 were reviewed: 35 had bilateral CTS. In all 10 on whom median nerve latency was measured, CTS was confirmed. Thirty-four of the 35 were considered on clinical and/or laboratory grounds to have active acromegaly. In 14 of the 15 tested, human growth hormone (HGH) assay was elevated. In the entire series (100), acromegaly was considered active in 64, inactive in 17 and indeterminate in 20. Applying the $\chi^2$ test the percentage of patients with and without CTS were statistically compared; a p value of 0.001 indicates overwhelming likelihood.
that acromegalic patients with CTS have active acromegaly when compared with acromegalics without CTS.

Pituitary irradiation (range 3,600 to 7,000 rads by linear accelerator or Cobalt"*) led to early relief of CTS in 2 but failed in 8. In the 8 failures, acromegaly remained persistently active; whereas, in the 2 whose CTS cleared after pituitary irradiation, acromegaly had become inactive. By contrast, hypophysectomy cured CTS in 5 and failed in 1; in these 5 acromegalic activity ceased by clinical and/or HGH assay; whereas, in 1 with continuing CTS, HGH activity was not suppressed. Spontaneous remission of CTS occurred in 3 patients, even though 2 showed persistently active disease.

Since the 2 hypotheses proved correct, we conclude that CTS in acromegaly is a useful indicator of active disease and that any treatment which abolishes hypersomatotropism relieves CTS. The finding that hypophysectomy appeared superior to conventional radiotherapy in treating active acromegaly is in keeping with the modern concept of optimal treatment of the disease. If, however, hypophysectomy is not feasible in active acromegaly with CTS, bilateral transverse carpal-ligament section is indicated.

Defective Phagocytosis in Patients with Systemic Lupus Erythematosus (SLE)

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Increased susceptibility to infections in patients with SLE is well known. Phagocytosis has been implicated as an important mechanism of defense. Therefore, we have studied in vitro phagocytosis in SLE and controls using the method of Hirsch and Strauss (E coli). Sixty-two percent of patients with active SLE showed markedly decreased phagocytosis. Their leukocytes phagocytosed normally in the presence of fresh normal serum. However, their sera failed to support normal leukocytes in phagocytosis, indicating a defect in the opsonic capacity of SLE serum. Since serum complement (C') participates in opsonization, measurements of opsonic capacity were correlated with those of serum #beta;1c-#beta;1A, C'. Results are shown:

Decreased phagocytosis was demonstrated in 8 of 13 patients with active disease, and in all of 6 with concomitant infections. The average opsonic capacity (−Δ log No. bacteria) of the active SLE patients with infection was lower than that of all other groups (p < 0.01).

Opsonization by nonimmune serum may depend on the presence of both serum C' and "natural antibody." Since both may be decreased in SLE, experiments were carried out to determine whether the low serum C' was responsible for the low opsonic capacity found. The addition to deficient sera of the 19s fraction of normal serum or of serum absorbed with antigen-antibody complexes did not restore phagocytic function, while addition of untreated serum resulted in partial restoration, suggesting that low C' was a limiting factor. It was concluded that the low serum C' was the limiting factor in the decreased serum opsonic capacity. The increased susceptibility to infection in active SLE may be explained by the above findings.

| Opsonic capacity (No. Normal/Total) | Normal controls | Inactive SLE | No infections | With infections | Miscellaneous infections |
|------------------------------------|----------------|-------------|--------------|----------------|-------------------------|
| #beta;1c-#beta;1A (mg%)            | 20/20          | 8/9         | 5/7          | 0/6            | 4/4                     |
|                                   | 190            | 149         | 120          | 88             | 178                     |

Relations Among 11 Laboratory Tests for Systemic Lupus Erythematosus (SLE)

DONALD W. PALMER, ULRICH H. RUDEFSKY, VADIM ORMISTE, WALTER R. WALLINGFORD, RAYMOND W. STEBLAY and JOHN S. THOMPSON, Chicago, Illinois

Eleven tests were performed on 13 patients un-results and which were independent. The tests deriving evaluation for possible SLE and compared were: (1) direct immunofluorescent staining (DIF) by χ² analysis to determine which gave similar of patients skin biopsies for IgG and C'3 at the
dermal-epidermal junction, or (2) in (DIF) epidermal nuclei, (3) serum complement activity (C') by both C'1 and immune adherence, (4) LE cell preparation, (5) calf thymus DNP-latex flocculation (Hyland LE test), (6) SS DNA-bentonite flocculation, (7) indirect nuclear immunofluorescence (ANIF) using as substrates for patients' sera, renal tubular nuclei in human kidney sections, (8) human lymphocytes, (9) human granulocytes, (10) chicken erythrocytes (RBC) and (11) mouse liver sections for peripheral, speckled, or diffuse nuclear staining pattern.

There was a significant association (p < 0.05) of low C' with DNP-latex, DNA-bentonite and chicken RBC ANIF. DNP-latex was associated (p < 0.05) with DNA-bentonite and chicken RBC ANIF. These 4 tests were positive in sera producing a peripheral pattern of ANIF and negative in sera producing a speckled pattern (p < 0.05). They also appeared to be associated (NS) with human kidney ANIF and DE junction DIF.

Human lymphocyte ANIF had a positive association (p < 0.05) with speckled staining and a negative association (p < 0.05) with low C', DNP-latex, DNA-bentonite, chicken RBC ANIF, and peripheral staining. Thus patients could be positive for either human lymphocyte ANIF or the C' related tests but not both. DE junction DIF, human kidney ANIF, human granulocyte ANIF, diffuse staining and clinical SLE appeared in both types of patient.

If DE junction DIF was taken (based on other reports) as the best test, the others could be ranked by $\chi^2$ in order of decreasing correlation with DE junction DIF in this highly selected group as follows: human kidney ANIF (4.1), low C' (2.6), DNP-latex (2.6), epidermal nuclear DIF (1.8), chicken RBC ANIF (1.4), human granulocyte ANIF (0.85), DNA-bentonite (0.31), LE prep (0.25), human lymphocyte ANIF (0.10), peripheral staining (0.08). These results do not necessarily reflect ability to separate SLE from other disorders.

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**Serum and Synovial Fluid IgG, IgA and IgM Anti-γ Globulins in Rheumatoid Arthritis (RA)**

Richard S. Panush, Nicolas E. Bianco and Peter H. Schur, Boston, Massachusetts

IgM antibodies to γ globulin have been emphasized in patients with RA; recently IgG and IgA anti-γ globulins (anti-IgG) have received attention. In this study sera from normal individuals and sera and synovial fluid (SF) from patients with osteoarthritis (OA) and RA were examined for IgG, IgA and IgM anti-IgG. Specimens were reacted with insolubilized human IgG; anti-IgG's were eluted with pH 2.8 glycine buffer, neutralized, and assayed for IgG, IgA and IgM content by radial immunodiffusion.

IgG and IgA anti-IgG were detected in normal sera, and in OA and RA sera and SF. Serum levels were higher in RA's than OA's, being greatest in latex positive patients. SF levels were higher in RA's than OA's. IgM anti-IgG were elevated in sera and SF of latex positive RA patients. RA patients with IgG or IgA anti-IgG above 2 SD of the mean of normals differed from RA's with "normally" distributed values by having more joint inflammation and destruction, prolonged and unremitting course, higher frequency of nodules and vasculitis, lower serum and SF complement levels and greater elevation of other anti-IgGs. They were also older. It is concluded that, in addition to IgM anti-IgG, IgG and IgA anti-IgG's are elevated in patients with RA, with the highest serum levels found in those with severe illness.

| Anti-γ Globulins (mean value μg/ml) |
|-----------------------------------|
| Serum                             | Synovial fluid               |
| No.     | IgG | IgA | IgM | No.     | IgG | IgA | IgM |
| Normals | 50  | 98  | 52  | <20   | 18  | 75  | 58  | <20  |
| OA      | 41  | 90  | 58  | <20   | 18  | 75  | 58  | <20  |
| RA      | 143 | 157 | 89  | 74    | 52  | 157 | 83  | 54   |
| latex negative | 68  | 113 | 77  | <20   | 24  | 143 | 83  | <20  |
| latex positive | 75  | 210 | 102 | 139   | 28  | 163 | 83  | 99   |

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Depression of Bone Marrow Granulocyte Reserves in Systemic Lupus Erythematosus (SLE)

HAROLD E. PAULUS, RONALD OKUN and JOHN J. CALABRO, Los Angeles, California

Bone marrow granulocyte reserves were estimated by measuring the increment of peripheral blood granulocytes per cumm after intramuscular etiocholanolone (0.1 mg kg) in 36 patients with SLE and 12 with chronic glomerulonephritis (CGN) who were being treated for nephritis with an immunosuppressive drug and/or prednisone. Leukocyte counts were done monthly; drug dosage was adjusted if leukopenia (WBC < 4,000/cu mm) was found.

Leukopenia during routine monthly visits was temporally related to 49 (mean granulocyte increment = 1901/cu mm) of 125 studies of the granulocyte response to etiocholanolone (GRE), while 76 studies (mean GRE = 5498/cu mm) were not associated with leukopenia. This association occurred in 46 of 99 studies in patients with SLE, but in only 3 of 26 with CGN. With GRE < 2,000/cu mm, all patients became leukopenic if given azathioprine or methotrexate. Leukopenia was associated with 21 of 26 studies in which GRE was between 2,000 and 2,800/cumm, contrasted with only 5 of 74 studies with GRE > 2,800/cu mm.

In all therapeutic groups the GRE was smaller in patients with SLE:

| Treatment      | CGN  | SLE  |
|----------------|------|------|
| Azathioprine   | 7379 (6) | 3336 (13) |
| Prednisone     | 4023 (4) | 3706 (22) |
| Both (p < 0.01) | 7089 (12) | 3696 (46) |
| Neither        | 5440 (4) | 2532 (9) |

larger if treated with azathioprine alone than if treated with neither agent. This may indicate that, for these patients, the therapeutic benefit of azathioprine more than compensated for its depressant effect on granulocyte production. With azathioprine alone, 154 mg/day was tolerated in CGN, contrasted with a daily average of 79 mg in SLE (p < 0.05); with combined azathioprine and prednisone therapy, 101 mg in SLE and 194 mg in CGN were given daily (p < 0.01).

This study demonstrates that SLE decreases marrow reserves of granulocytes. The dose of azathioprine tolerated by patients with SLE was smaller than the dose tolerated by patients with CGN. However, if the GRE was used to monitor therapy, immunosuppressive dosage could be individualized and maximum tolerated doses could be given.

Plasma Infusions in a Patient with Selective IgA Deficiency and Juvenile Rheumatoid Arthritis (JRA)

ROSS E. PETTY, JAMES T. CASSIDY, ANN BURT and DONITA B. SULLIVAN, Ann Arbor, Michigan

There is an established relationship between connective tissue diseases and immunoglobulin deficiency states. Previous studies have reported amelioration of the arthritis associated with agammaglobulinemia after treatment with intramuscular γ globulin. More recently, human plasma as a source of antibody has been advocated as an alternative mode of treatment.

In the present study, the effect of plasma infusions was evaluated in a 9-year-old girl with selective IgA deficiency, repeated respiratory infections and JRA. Absence of IgA in serum, saliva and tears was verified by radial and double diffusion in agar with monospecific antisera. Five plasma infusions (700-1100 mg IgA) were administered during a 7-month period. One infusion of 5% human albumin was given as a control. Serial measurements of serum immunoglobulins, secretory IgA, C-reactive protein, rheumatoid factor and antinuclear antibodies were performed and correlated with disease activity as estimated by the joint index.

Three significant observations were made. First, the joint index decreased 2-5 days after each plasma infusion but was unchanged after the albumin. The degree of synovitis has remained less than it was prior to the study, and there has been no further evidence of infection. Second, although there was no detectable IgA prior to the first infusion, small amounts of serum IgA (0.04 mg/ml) are present 11 months after the last infusion. The observed half-life of serum IgA increased from 5-7
ABSTRACTS
days after the first infusion to 9-14 days. Anti-IgA antibodies have not developed. Third, lymphocyte transformation of peripheral blood leukocytes after phytohemagglutinin stimulation increased from 27% to a normal value of 87%.

It is worthy of note that plasma infusions in this patient with selective IgA deficiency were followed by the observed modulations of both the humoral and cellular aspects of immunity and the clinical inflammatory state.

The Hypothalamus-Anterior Pituitary-Cortical Adrenal-Lymphoid Tissue Endocrine System and Its Role in Immunity
Nicolau Radoiu and Frederick A. Zydeck, Detroit, Michigan

ACTH and adrenal corticoids have a dramatic and favorable effect in allergic states and in collagen diseases. These hormones also cause a decrease and dissolution of lymphocytes with release of lymphocytic cellular constituents. These observations suggest that the favorable effect of ACTH and corticosteroids in hypersensitivity and in the diseases of the connective tissue might be mediated by some components of the lymphoid tissue. This would also imply that lymphoid tissue represents a target organ in a hypothalamus-anterior pituitary-cortical adrenal-lymphoid (?endocrine) system.

To test our hypothesis, we studied the effect of a crude lymph node extract on immunity and hypersensitivity, using as models diphtheria immunization in guinea pigs, horse serum-induced immediate hypersensitivity in rabbits, BCG-induced delayed hypersensitivity in guinea pigs, and also the effect of the lymph node extract on plasma and urine corticosteroids.

Our experiments suggest an interference of lymphoid tissue with the process of immunization and hypersensitivity. They also indicate suppression of the adrenal cortical steroids by the lymphoid tissue, suggesting that lymphoid tissue could represent a target gland in a hypothalamus-anterior pituitary-cortical adrenal-lymphoid system. It might be possible that some of the therapeutic effects attributed to corticotropin and corticosteroids are, in fact, due to some of the cellular components of the lymphoid tissue.

Antigenic Abnormalities of Serum IgG in Rheumatoid Arthritis (RA)
Arnold J. Rawson and Neva M. Abelson, Philadelphia, Pennsylvania

It has been shown by Hollander and coworkers that the introduction of IgG into the quiescent joint of a rheumatoid arthritic induces a severe reaction only if the IgG is obtained from a rheumatoid donor. Utilizing the finding of Henney and Ishizaka that the guinea pig (GP), by the formation of precipitating antibody (Ab), is capable of distinguishing antigenic groupings of aggregated IgG (AGG) from those of native IgG, we have designed experiments to explain this observation.

Whole serum from rheumatoid or normal donors was used as a source of IgG. Whole serum, 0.05 ml, emulsified in complete Freund's adjuvant, was injected into the rear footpads of outbred female GP, using 4 GP for each serum sample. The injections were repeated at 2 and 4 weeks, substituting incomplete for complete adjuvant. The animals were bled 4 weeks later. Each of the sera from rheumatoids evoked an Ab response to AGG. This was found in 7 of 11 GP. Seven of 11 GP in this same group had Ab against unaggregated IgG. Of the surviving GP which received serum from normal donors, none of 13 had Ab against AGG; only 3 of 13 had Ab against unaggregated IgG.

In a second series of GP, 1 mg IgG from rheumatoid or normal donors, isolated by DEAE-cellulose, was emulsified in complete Freund's adjuvant and injected into the rear footpads. The animals were bled at 4 and 7 weeks. At 4 weeks, Ab to AGG was found in 46% (12/26) of those receiving IgG from rheumatoids and 32% (7/22) of those receiving IgG from normals, while Ab to IgG was found in 62% (16/26) and 77% (17/22), respectively. After 7 weeks, no differences were seen between the two groups.

The findings suggest the presence in RA either of circulating complexes or of an abnormality of IgG which facilitates exposure of AGG antigen.
**Scanning Electron Microscopy (SEM) of Human Articular Cartilage**

IRVING REDLER and MARILYN L. ZIMNY, New Orleans, Louisiana

Normal and pathologic cartilage was studied by SEM to ascertain morphologic characteristics. Specimens obtained during surgical procedures were fixed in gluteraldehyde, dehydrated in graded alcohols and coated with a thin layer of carbon and gold-palladium alloy in a vacuum evaporator. Articular surfaces as well as sagittal and tangential sections through the cartilage were examined in Cambridge and JSM-V5 scanning microscopes. The striking differences in surface and subsurface structure revealed by SEM will be illustrated by lantern slides and will be correlated with transmission electron microscopy of the same specimens.

**Hemolytic Measurement of the Ninth Complement Component: Evidence for Completion of Complement Reaction Sequence in Human Disease**

SHAUN RUDDY, PETER H. SCHUR and K. FRANK AUSTEN, Boston, Massachusetts

The ninth and terminal component of complement (C9) accelerates the production of cytotoxic defects in cell membranes initiated by the first 8 components of the complement sequence. C9 activity contained in serum and other biologic fluids was measured by the lysis of sheep erythrocytes which had been sensitized with antibody and reacted with the first 8 complement components.

In vitro, C9 is consumed during completion of the complement reaction sequence. In the present study, analogous depletion of C9 in vivo, presumably by immunologic reactions occurring either systemically or locally, has been observed. In some patients with systemic lupus erythematosus (SLE), C9 levels were elevated above normal during periods of relative quiescence of the disease and fell precipitously with the onset of clinically apparent renal involvement. These C9 changes were often preceded for several months by depressions of other earlier reacting components (C1, C4, C3). In some SLE patients with equally marked reductions of the early components but without renal disease, the C9 levels were normal. Depressions of serum C9 were also observed in a patient with "hypocomplementemic" nephritis (normal C1, C4, low C3), indicating utilization of the terminal steps of the complement sequence in this disease as well.

Although serum levels of C9 among patients with seropositive rheumatoid arthritis (RA) were similar to those of patients with seronegative RA, synovial fluid levels of this component were significantly lower in seropositive disease. These results indicate that the activation and fixation of C1, C4, C2 and C3 previously observed in seropositive RA is associated with completion of the cytotoxic reaction sequence in the joint space.

**Cutaneous Alterations in Progressive Systemic Sclerosis Following Administration of Antilymphocytic Globulin (ALG)**

ARTHUR L. SCHERRER and LAWRENCE J. McCORMACK, Cleveland, Ohio

Antilymphocytic globulin, known to prolong survival of homografts, is presently being evaluated in 2 patients with progressive systemic sclerosis. After receiving ALG for approximately 1 year, both patients show significant objectively documented softening of skin and subcutaneous tissue. Light microscopy of skin biopsies before treatment in both patients showed dense collagen in all of the dermis seen. After 3 to 6 months of ALG administration, looseness of sclerodermic collagen appeared in the superficial dermis with the return of normal acid mucopolysaccharide distribution.

The electron microscopic changes paralleled and enhanced the light microscopic findings. Prior to administration of ALG the patients with scleroderma showed densely packed collagenous fibers immediately adjacent to the cytoplasm of the fibroblast. No paracellular spaces were seen around the fibroblasts, and no precollagen was recognized. After ALG administration, individual fibroblasts acquired a more active appearance with a variable increase in electron density, Golgi apparatus, endoplasmic reticulum and secretory vacuoles. The collagenous matrix was now separated from fibroblastic cell bodies. Masses of degenerating collagen containing new collagenous fibers and aggregates of material considered to be precollagen surround the fibroblasts. At one stage, these collagenous fibers were quite variable in diameter.

It appears that ALG therapy has restored control.
ABSTRACTS

by the fibroblast over collagenolysis. One may speculate from these findings that the defect in scleroderma concerns inability of the fibroblast to break down collagen as part of a dynamic equilibrium. If an immune mechanism exists, it must be operating at this level of collagen breakdown. ALG must aid in the return of the ability of the fibroblast to control the breakdown of collagen.

Hyperuricemia in Branched Chain Ketoaciduria (Maple Syrup Urine Disease)

JOSEPH D. SCHULMAN, THOMAS J. LUSTBERG and J. EDWIN SEEGMILLER, Bethesda, Maryland and La Jolla, California

A renal retention of uric acid can be induced by infusion of a variety of organic acids that are intermediates of normal metabolism. Metabolic derangements leading to accumulation of such compounds as lactic, acetoacetic and \( \beta \)-hydroxybutyric acids are also accompanied by hyperuricemia.

A branched chain ketoaciduria was found in a girl aged 19 months, presenting with mental retardation (developmental age of 7 months). She manifested a previously undescribed variant of maple syrup urine disease characterized by an incomplete deficiency of the decarboxylase activities for the ketoacids derived from leucine, isoleucine and valine and a constant amino- and ketoaciduria. This variant differs from classical maple syrup urine disease in the mildness of neurologic dysfunction and the survival of the patient without treatment.

A serum urate of 9.8 was present on an unrestricted diet with urinary uric acid/creatinine ratio of 0.6 and a 24-hr ketoacid excretion of 2480 mg. A low protein diet diminished the 24-hr ketoacid excretion to 78 mg and the serum urate to 7.2 mg/100 ml. Further restriction of intake by administering a semisynthetic diet diminished the ketoacid excretion to 20 mg/24 hr and the serum urate to 4.2 mg/100 ml, and increased the urinary uric acid/creatinine ratio to 1.1. Separate administration of valine, isoleucine or leucine resulted in an increase in ketoaciduria, a corresponding diminished renal excretion of uric acid, and an increase in serum urate to 6.2, 6.9 and 8.8 mg/100 ml, respectively. The degree of hyperuricemia was correlated with the amount of ketoacid excretion.

This child gave no history of joint symptoms but if untreated, the persistence of the degree of ketoaciduria and hyperuricemia initially present into adult life should place her at high risk for development of gouty arthritis. The possible presence of this new variant of maple syrup urine disease should therefore be considered in mentally retarded patients who present with marked hyperuricemia or gouty arthritis in later life.

Sequential Changes in Polymorphonuclear Leukocytes After Urate Crystal Phagocytosis: An Electron Microscopic Study

H. RALPH SCHUMACHER and PAULDING PHELPS, Philadelphia, Pennsylvania

Polymorphonuclear leukocytes (PMN's) and urates appear to be essential for the inflammation of acute gouty arthritis. We have examined the sequential ultrastructural changes following in vitro phagocytosis of monosodium urate crystals added to human leukocytes. Aliquots were fixed in \( \frac{1}{2} \) strength Karnovsky's paraformaldehyde-glutaraldehyde and osmium tetroxide at intervals of 3, 8, 30 and 120 min.

At 3 min, 10% of leukocytes had phagocytized crystals, all of which were surrounded by a phagosome membrane which was closely applied to the crystal surface. At 8 min intact dense bodies and granular material had been released into many phagosomes. Some crystals were more widely separated from the phagosome membrane (apparently by fluid), while in other instances portions of crystal borders seemed to be in direct contact with the PMN cytoplasm. At 30 min some crystal laden cells showed areas of decreased cytoplasmic density (possibly a result of degranulation) and other cells were necrotic. By 2 hr 50% of PMN's had phagocytized crystals. In addition to the changes seen in earlier specimens, occasional crystals seemed to lie entirely free in the cytoplasm of viable cells. Only portions of phagosome membranes could be defined around other crystals. Cell necrosis was more frequent.

Control cells not exposed to crystals, and exposed cells which had not phagocytized crystals did not show these changes. Crystals were also phagocytized by mononuclear cells and platelets.

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These results show rapid progression to cell death in some PMN's following urate crystal phagocytosis. The loss of phagosomal membranes may be important in the cell damage. The early close contact between the crystal surface and the phagosomal membrane suggests a possible direct lytic effect of the crystal on the membrane. Distention of some phagosomes with fluid and granular material may also indicate contributions of enzymatic or osmotic factors. The sequential disappearance of phagosomal membranes seen here after crystal phagocytosis suggests that the absence of membranes surrounding crystals need not imply that the crystal was formed intracellularly.

Serologic and Virologic Studies in Patients with Rheumatoid Arthritis (RA)

James P. Simsarian, Henry Roth, Hope E. Hopps, Robert D. Douglas, Michael S. Williams and Harry M. Meyer, Jr, Bethesda, Maryland and Washington, DC

In a search for viral agents associated with RA, joint fluids, synovial biopsies and sera were examined. Serologic screening showed that 7 of 27 patients with RA (26%) had complement fixing (CF) antibodies for Herpes simplex as compared with 31 of 33 non-RA patients (94%) in the same clinic. When RA sera with a Bentonite flocculation titer (BFT) of 1:1024 and no Herpes simplex antibody were added in equal volumes to BFT-negative sera with Herpes simplex antibody titers of 1:8 to 1:64, a depression of Herpes simplex CF antibody was noted. The observed titers were lower than could be accounted for by dilution alone. No differences were observed between control and RA patients in varicella-zoster and mumps CF antibody titers, and in mumps, rubella and measles hemagglutination-inhibition antibody levels.

Twelve cell cultures established from RA joint fluids or synovial biopsy material and 8 control cultures were studied by cocultivation of cultured cells with human and simian cells, observation for cytopathic changes, tests for hemadsorbing-hemagglutinating agents and inoculation of mycoplasma culture media. Twenty-one joint fluids from 14 RA patients were inoculated on human and simian cell lines. No viruses or mycoplasmas were isolated.

Joint fluids from 7 of 11 RA patients and 3 (1 patient with Reiter's syndrome and 1 with an undefined arthritis) of 11 control patients were inoculated on human and simian cell lines. No viruses or mycoplasmas were isolated.

Tetracycline in the Treatment of Rheumatoid Arthritis:
A Double-Blind Controlled Study

Martha Skinner, Edgar S. Cathcart, John A. Mills and Robert S. Pinals, Boston, Massachusetts

Despite increasing speculation that microorganisms, such as mycoplasma or diphtheroids may play an etiologic role in rheumatoid arthritis, controlled studies of the effectiveness of long term antibiotic treatment have not been reported. For this reason, a study was undertaken to evaluate a 1-year trial of low dose tetracycline therapy in a double-blind manner.

Twenty-seven patients with definite or classic rheumatoid arthritis were selected from 3 university hospital arthritis clinics and randomly assigned to treatment with either tetracycline, 250 mg/day, or a placebo. The patients were evaluated by the same physician on 9 consecutive visits and the following parameters were measured: grip strength, number of tender and swollen joints, ring size, walking time, duration of morning stiffness, sedimentation rate and range of motion of each joint. The data were analyzed (1) by comparing the mean values for each parameter before and after treatment in each group and between the two groups and (2) by deriving a final score for each patient based on the results in all parameters.

Of 13 patients in the tetracycline group, 3 improved; 7 remained unchanged; and 3 became worse. Of 14 patients on placebo, 5 improved; 6 remained unchanged; and 3 became worse. Both groups tended to lose joint motion over the 1-year period. Morning stiffness increased in the tetracycline group and was significantly worse when compared either to initial values or to the placebo group. Other parameters did not differ significantly.
ABSTRACTS

Three synovial fluids were assayed for tetracycline and found to contain 56-78% of the serum values. This study clearly demonstrated no significant benefit from long-term tetracycline therapy at a dose of 250 mg/day in patients with rheumatoid arthritis.

Decreased Synthesis of the Third Component of Complement in Hypocomplementemic Systemic Lupus

Anthony J. Sliwinski and Nathan J. Zvaifler, Washington, DC

Serum complement is often low in patients with systemic lupus erythematosus (SLE), presumably due to increased utilization by immune complexes. The catabolic (Km) and synthetic (Ks) rates of the third complement component (C′3) were determined in normal subjects and 9 patients with SLE. Six of the 9 patients were hypocomplementemic; 4 of these had active renal disease. In 6 normals, the Km was 1.8 to 3.5%, and Ks 0.84 to 2.0 mg/kg/hr. Two SLE patients with normal C′3 had a high Km and Ks, one with increased C′3 had a high Km and Ks. Four patients with untreated SLE and depressed C′3 had Km of 5.3%, 1.6%, 2.3% and 3.5%, with Ks of 0.48, 0.51, 0.74 and 0.95 mg/kg/hr, respectively; 2 others with low levels taking steroids had normal Km, but depressed Ks.

Two of the 4 untreated patients with depressed C′3 were restudied. After steroid treatment, both had a decrease in Km; 1 had no change in Ks with an increase of C′3 from 21 to 42 mg%, and the other had a change in Ks from 0.74 to 0.87 while C′3 went from 50 to 100 mg%. In summary, 5 of 6 SLE patients with low C′3 had a depressed Ks while only 1 had marked increase in Km with a normal Ks. The lowest values of C′3 were generally associated with the lowest Ks. Depressed Ks occurred with or without renal disease.

These results indicate that static measurements of C′3 do not quantitate the degree of C′3 utilization. In fact, the major determinant of the low C′3 observed in this study of SLE was decreased synthesis of C′3.

Changes in Rabbit Joints Injected with Rheumatoid Synovial Membrane Cells

Carol Smith, Rosamond Janis, Edward Haberman and David Hamerman, Bronx, New York

Rheumatoid synovial cells in culture possess several characteristics that distinguish them from cultured cells derived from nonrheumatoid membranes. In particular, the rheumatoid cells are resistant to Newcastle disease virus and rubella virus; the nonrheumatoid cells are susceptible to both. These findings support the suggestion that a latent viral infection may persist in the rheumatoid synovial cells (Arthritis Rheum 12:639, 1969). Studies were done to see if joint changes observed in rheumatoid arthritis could be produced in animals by intra-articular injection of cultured rheumatoid cells. To date, 8 rabbits have been examined: in 2, both knees were used, 1 knee receiving rheumatoid cells and the opposite knee nonrheumatoid cells (controls); in 6, only 1 knee was injected, 3 receiving rheumatoid cells and 3 nonrheumatoid cells (controls). Two to 6 months after injection, histologic evaluations were made on each pair of rabbit joint tissues. Based on criteria compatible with rheumatoid synovitis, differences were detected between 4 of the 5 pairs of rabbit joints: the more severe histologic changes were found in the joints previously injected with rheumatoid cells.

Cell cultures derived from the synovial membranes obtained from each of these injected rabbit joints were challenged with rubella virus. The control rabbit cell cultures died within 10 days; the rabbit cell cultures from joints injected with rheumatoid synovial cells were resistant.

These animal studies indicate that properties of rheumatoid synovial cells persisting in culture can be transmitted to normal rabbit synovial membrane cells.

The Role of Thermography in the Evaluation of Peripheral Joint Inflammation

Charley J. Smyth and Frank Kreith, Denver, Colorado

Skin temperature over inflamed joints has been measured using specially designed thermocouples and thermistors that are sensitive to 0.1 C° to 0.01 C° respectively. Variations in patients with acute synovitis and in control subjects with exercise, bed rest, time of day, dietary intake and both room
and body core temperature have been determined. Eight patients with acute gouty arthritis were studied. The difference in skin temperature over the inflamed joint as compared to the uninvolved paired joint ranged from 2.0 to 6.2°C. Serial measurements in gouty patients correlated with other objective parameters of inflammation and paralleled clinical response to therapy.

Fourteen rheumatoid patients had differences over actively inflamed peripheral joints (wrists, elbows, metacarpophalangeal, proximal interphalangeal, knees and ankles) that ranged from 0.5 to 2.5°C. Eight normal individuals served as controls. Measurements taken under a variety of conditions showed differences varying from 0.2 to 1.0°C.

These observations indicate that thermography using thermistors and thermocouples is a reliable means of recording joint inflammation.

**Suppression of Experimental Allergic Encephalomyelitis (EAE) by Methotrexate (MTX) with Folinic Acid (FA) Rescue**

Murray C. Sokoloff and Brian B. Newbould, Los Angeles, California

The use of MTX in suppressing immunity is limited by its toxicity. Delayed FA rescue can decrease the toxicity and leave the immunosuppressive effects intact. The rescue technic has not been tried in autoimmune disease. We therefore evaluated the suppressive effects of MTX alone and MTX with delayed FA on EAE in CFN rats.

Preliminary work showed that MTX was least toxic when given in a single intravenous (IV) injection to CFN rats, the LD₅₀ being about 150 mg/kg. When 60 mg/kg was given weekly, the drug was uniformly fatal after the second or third injection. However, when FA, 100 mg/kg, was given up to 8 hr after each MTX injection, there was complete protection from toxicity.

CFN rats (150–200 g) were given intra-lymph node injections of guinea pig spinal cord emulsified in complete Freund's adjuvant. EAE occurred within 8–14 days in untreated animals. Single doses of MTX up to 140 mg/kg with and without FA rescue had only minor effects on the subsequent EAE. MTX, 60 mg/kg, plus FA 8 hours later begun on Day 2 after AG and given weekly thereafter, resulted in very mild, transient disease with onset averaging Day 21 in 2 of 8 as opposed to severe persistent disease with onset averaging Day 12 in 10 of 12 saline-treated controls. MTX was stopped after Day 30 in 4 animals and Day 59 in 4 others with no subsequent EAE. When weekly MTX plus FA was begun Day 8 after AG, there was complete suppression of EAE in all animals.

It appears that the FA rescue technic can effectively counteract the toxicity of MTX while not significantly diminishing its effectiveness in the therapy of EAE.

**Cytotoxic Reaction of Serum from Patients with Systemic Lupus Erythematosus (SLE) with Allogeneic and Autologous Lymphocytes**

Peter Stastny and Morris Ziff, Dallas, Texas

Using a standard microassay employed for HL-A antigen typing, sera from 60 SLE patients were tested for cytotoxicity in the presence of rabbit complement against a panel of 36 lymphocytes, obtained from either normal individuals (18) or patients with SLE (18). A 2+ or greater cytotoxic effect was produced by 60% of SLE sera. Three groups of control sera were also investigated. These were as follows: 18 sera from blood bank donors; 45 control sera matched with the SLE group for age, sex, race, and conditions of storage; and 366 sera from women with 4 or more pregnancies. Cytotoxic reactions were observed in 0%, 4%, and 12%, respectively, of the control groups.

Since the frequency of cytotoxicity for allogeneic lymphocytes was much higher in SLE patients than in the controls, experiments were performed to determine whether reactions also occurred with autologous cells. When lymphocytes from 20 SLE patients were incubated with from 1 to 22 samples of their own sera, 11 patients (55%) showed an autologous reaction. This was, however, not continuously present. Sera from only 3 patients demonstrated cytotoxicity against the lymphocytes of the blood samples from which they were obtained.

Experiments are underway to investigate the possible antibody nature of the cytotoxic factor found in SLE sera and to identify the membrane
Controlled Trial of Cyclophosphamide in Systemic Lupus Erythematosus Nephritis

Alfred D. Steinberg, H. Benfer Kaltreider, Parker J. Staples, Edward J. Goetzl,
Norman Talal and John L. Decker, Bethesda, Maryland

Eleven women with lupus nephritis were hospitalized for a 10-week double-blind therapeutic trial comparing oral cyclophosphamide (Cy) with placebo (P). Random assignment put 7 patients on Cy and 4 on P. Two P patients were subsequently treated with Cy by the same protocol, and are included in both Cy and P groups. Cyclophosphamide dosage averaged 2.2 mg/kg/day (1.3-2.9), the exact dose depending upon the extent of leukopenia. Concurrent corticosteroid therapy at a steady dose of prednisone of 30 mg/day or less was permitted.

Seven of 9 Cy patients and 3 of 4 P patients received prednisone. Pretreatment renal biopsies in 6 patients showed glomerulitis in 1 and glomerulonephritis with hyalinization and variable hypercellularity in the others.

Two Cy patients did not complete the study. One died of pulmonary embolism in Week 5. The other, on corticosteroids and salicylates, sustained a gastrointestinal hemorrhage in Week 6. Other complications in Cy patients included alopecia in all, hemorrhagic cystitis in 1 (Week 8) and a pseudomonas axillary abscess in 1 (Week 6). There were no unusual developments in the P group.

All Cy patients developed absolute lymphopenia and major reductions of immunoglobulins G and M if these were initially elevated. Results of the 13 courses of therapy are shown:

|         | Cy | P |
|---------|----|---|
| Increased creatinine clearance | 3/9 | 1/4 |
| Improved urinary sediment | 4/9 | 0/4 |
| Reduced 24 hour urine protein | 6/9 | 1/4 |
| Rise in serum complement (C'3) | 6/9 | 0/4 |
| Fall in anti-DNA antibodies | 7/9 | 0/4 |
| Improved extrarenal disease | 5/9 | 0/4 |

The best responses were seen in the patients with the mildest renal disease. Two of the mildest received both P and Cy courses: of the 6 listed parameters, 1 patient showed 1 better on P and 6 better on Cy; the other had 0 better on P and 5 better on Cy.

It is concluded that cyclophosphamide, although toxic, can bring about an improvement in some patients with lupus nephritis. Means for increasing the efficacy and reducing the toxicity of the drug should be sought.

Enhanced Plaque-Forming Cell Response to S-RBC in Adjuvant Arthritis

C. A. L. Stephens, Jr, James L. Parsons and A. B. Stanfield, Tucson, Arizona

As part of a continuing investigation of lymphoid cells, this study was undertaken to determine whether rats injected with Mycobacterium butyricum in oil were also capable of response to a later injection of S-RBC.

Sprague-Dawley and Holtzman strain rats (180-120 g) were used. Adjuvant mixture (0.5 mg M butyricum/rat) was injected intracutaneously in the tail on Day 0 according to technics established by Waksman et al. On Day 4, washed S-RBC (1 X 10^9) were injected intravenously in one group of animals. Spleens were removed on Day 8 and the animals kept for further observation. Splenic cells were teased into Eagle’s MEM, and the response of each animal to S-RBC was assayed by the Jerne plaque-forming cell (PFC) technic.

Data show that rats which received both adjuvant and S-RBC exhibited enhanced FCP response over those which received S-RBC alone. The ratio of response was 1:3.4 (Sprague-Dawley) and 1:2.55 (Holtzman). Background plaquing was not observed in the rats which received M butyricum in oil without S-RBC, nor in the noninjected controls.

In the adjuvant + S-RBC groups, compared with the groups receiving the adjuvant only, onset of joint involvement occurred earlier, and the severity of inflammation was more intense throughout the 21 days of the experiment.

M butyricum in oil given 4 days before challenge with S-RBC markedly increased PFC response and the challenge of S-RBC intensified and accelerated joint inflammation. These data suggest that adjuvant arthritis may represent a hyperimmune reactive disease state.
Urinary Fibrinogen Fragments in Lupus Nephritis
Mary Betty Stevens, Thomas M. Zizic and Neal Young, Baltimore, Maryland

Antigen-antibody interaction and fibrin deposition are capable of stimulating formation of plasmin, a fibrinogenolytic and fibrinolytic factor which also may contribute to tissue damage through activating the complement system. Thus, increased fibrinogenolytic activity might be expected in active lupus nephritis. Two hundred and seventy-four urine concentrates from 21 patients with SLE and 64 controls were evaluated by double diffusion and immunoelectrophoresis against rabbit antiserum to purified human fibrinogen. Fibrinogen fragments were demonstrable in 9 patients, namely, 5 of 12 with lupus nephritis, 2 of 2 patients with acute poststreptococcal glomerulonephritis, and 2 of 2 patients with renal allografts. Patients with nonrenal inflammatory disorders (including SLE) and nonimmunologic renal disease had negative urines. The presence of urinary fibrinogen fragments correlated with active lupus nephritis, clinically and histologically. Furthermore, in patients with lupus nephritis, serum complement was depressed in 4 of 5 patients with urinary fibrinogen fragments, but only in 2 of 7 with negative urines. Fibrinogen fragments were not detectable in either serum or plasma. These data show an increase in renal fibrinogenolysis (or fibrinolysis) in active lupus nephritis and may provide an important index of renal inflammation as well as have pathogenetic implications.

The Rheumatic Spectrum of Gonococcal Infection
John D. Stobo, Willis C. Maddrey, Louise T. Knoke, and Mary Betty Stevens, Baltimore, Maryland

Although gonococcal infections may present with a variety of clinical manifestations, the patterns of articular involvement have not been emphasized. Reported here are 70 patients with gonococcal arthritis, classified as definite (13 patients), probable (29 patients), or possible (28 patients), according to predetermined bacteriologic criteria. Joint involvement, which was predominantly polyarticular, occurred in 3 different patterns: (1) effusion, (2) effusion with tenosynovitis and (3) tenosynovitis alone. While 65% of the patients with only effusions had monarticular disease, 30% of the patients with tenosynovitis with or without effusions had a single joint involved. Although the knee was the predominant joint involved in effusive disease, the wrist was more commonly involved in patients with tenosynovitis. It is of interest that of those 15 patients with a previous history of arthritus, regardless of type, 13 had effusions.

Inappropriate Intersosseous Muscle Activity in the Rheumatoid Hand
Robert L. Sweezy and Donald S. Fiegenberg, Los Angeles, California

An abnormality of function of the intrinsic muscles associated with swan neck deformity and metacarpophalangeal subluxations in rheumatoid arthritis has long been appreciated, but not explained. No electromyographic evidence of spasm of these muscles nor consistent evidence of sufficient myositis to explain the increased intrinsic muscle tension has been observed. In 8 consecutive rheumatoid subjects with active metacarpophalangeal joint involvement, inappropriate intersosseous activity was demonstrated by electromyographic evidence of muscle contraction occurring at 10-30° of extension (0° = full exten-
sion) during active extension. No muscle action potentials were observed at more than 5° in the controls.

It is suggested that a protective metacarpopha-

**Autoallergic Diseases with Serum IgG Reactive with Vascular Structures**

ENG M. TAN, ROBERT T. REID and CARL M. PEARSON, La Jolla and Los Angeles, California

Many investigators have reported that vasculitis is commonly seen in dermatomyositis, polymyositis, rheumatoid arthritis and giant cell arteritis in polymyalgia rheumatica. With indirect immunofluorescence, we have looked for serum antibodies in different autoallergic diseases and have detected circulating γ globulin reactive with capillary structures in cryostat sections of frozen mouse kidney used as substrate. The most reactive site in mouse kidney sections is the peritubular capillary network, but glomerular capillaries and intima of arterioles are also reactive. By zone electrophoresis separation or density gradient ultracentrifugation analysis of several sera, the reactive serum factor was identified as IgG. The reaction between serum IgG and capillaries in mouse kidney sections was shown to fix complement by an immunofluorescence method demonstrating fixational C3 at capillary sites when fresh serum was used, but no fixation of C3 with decomplemented serum. The incidence of capillary reactive IgG in different diseases was: scleroderma 17/31 (55%), dermatomyositis 16/35 (46%), rheumatoid arthritis 13/41 (32%), systemic lupus erythematosus (SLE) 6/40 (15%) and “normal” blood donors 8/75 (11%). Six polymyalgia sera were available for study and the incidence was 3/6. All SLE sera and some scleroderma and rheumatoid arthritis sera contained antinuclear antibodies (ANA), and in instances where ANA and capillary reactive IgG were present together, there was both tubular nuclear and peritubular capillary staining by indirect immunofluorescence described above. In contrast, the majority of dermato-polymyositis sera showed only capillary staining and no nuclear staining. Capillary reactive serum IgG has also been found in 3 cases of temporal arteritis and 1 case of pulseless disease due to giant cell arteritis. In one temporal artery biopsy examined by immunofluorescence, in vivo fixation of IgG and C3 was demonstrated in the intima of small vessels in the adventitia but not in the intima of the temporal artery itself. These studies report the finding that in certain autoallergic diseases often associated with vasculitis or arteritis, circulating serum IgG has been identified which has some of the properties of antibody and is reactive in vitro with vascular structures. A similarly reactive IgG is present in lower incidence in “normal” blood donors.

**Assessment of Leukocyte Function in Systemic Lupus Erythematosus**

GARY E. TRATT, ALLEN R. MYERS, ROBERT C. MOELLERING, ARNOLD N. WEINBERG and KURT J. BLOCH, Boston, Massachusetts

Clinical experience suggests that patients with systemic lupus erythematosus (SLE) demonstrate an appreciable incidence of serious infections. Since polymorphonuclear leukocytes (PMNL) play an important role in the normal host defense against bacterial infection, an attempt was made to determine whether abnormalities in PMNL function might account for decreased resistance to infection in patients with SLE. In an attempt to assess certain functions of PMNL, peripheral blood samples were obtained from a control group and from 20 patients with active SLE (including 5 with a past history of serious infection).

Since normal PMNL rapidly reduce nitroblue tetrazolium (NBT) dye during in vitro phagocytosis, it has been suggested that the ability of PMNL to reduce dye spontaneously in vitro may reflect prior in vivo stimulation. In 20 patients with SLE, spontaneous reduction of NBT was seen in 3% of PMNL compared to an average of 7% in 12 controls. On challenge with zymosan particles, PMNL from both patients and controls demonstrated reduced NBT dye in 98% of the phagocytic cells. The ability to phagocytose and kill S. epidermidis was assessed in a system employing fresh autologous serum, peripheral white blood cells, fetal calf serum, bacteria and tissue culture medium. The number of viable organisms (colony-forming units) remaining after 3 hr incubation at 37°C was
determined. In 12 controls, the percent of viable bacteria varied from < 1 to 8; in 16 patients with SLE the percent of viable organisms also varied from < 1 to 8. These findings suggest that in the parameters tested, PMNL from patients with SLE function as well as control cells.

**Evaluation of Blood Counts in Rheumatoid Arthritis Treated with Alkylating Agents**

HAROLD C. TRETBAUL, Tucson, Arizona

The blood counts of 30 rheumatoid arthritics started on treatment with alkylating agents between July 1966 and July 1968 were reviewed. No adverse side effects were found. Treatment included nitrogen mustard, thiotepa, chlorambucil and cyclophosphamide. Maintenance therapy is usually oral cyclophosphamide 50-100 mg/day to keep the white blood cell count in the range of 3-4,000/cu mm.

A patient with Felty's syndrome increased his white count to normal and his splenomegaly disappeared. Five of the 30 patients were anemic with initial hemoglobin levels below 10.6 g/100 ml. There was no evidence of blood loss or hemolysis. No hematinics were added, but several patients were continued on iron started previously. A rise in hemoglobin above 13 g/100 ml was noted to parallel the general improvement of the arthritis. This usually occurred after 7-12 months of alkyla
tion.

One patient required a total of 56 transfusions over a 3-year period. After 1 year of treatment no further transfusions were required to maintain a hemoglobin level of 13 g/100 ml. This was maintained until his death 2 years later of amyloidosis.

This retrospective study has shown improved bone marrow response during prolonged alkylation. The reasons for decreased blood cells in active arthritis have not been clarified although there seems to be decreased erythropoietin production.

**A Rapid Method for Evaluation of the Structure and Function of the Rheumatoid Hand**

PAUL S. TREUHAFT, MARILYN R. LEWS and DANIEL J. MCCARTY, Chicago, Illinois

Numerous systems have been devised to quantify structural and functional abnormalities in rheumatoid joints. Most are complex and require intricate equipment as well as a great deal of time and energy. The rapid, simple and sensitive method described here permits study and evaluation of results of medical and surgical treatment and the natural history of the disease.

Each of the small hand joints is examined for ligamentous and capsular stability, integrity and anatomic position of tendons, and range of motion (approximated by inspection of the flexed and extended digits). Similar evaluations of the distal radioulnar and wrist joints are made. Grip strength and pinch strength are measured by a standard sphygmomanometric technic. Data are recorded on a life-sized hand outline form in simple abbreviation code. This form provides numerical data suitable for computerization and permits a retrospective reconstruction of the abnormalities present. An examination on a single patient is performed by a trained observer in 20 ± 5 min; 23 patients examined by two observers provided the data reported here. Duplicate examinations were made by the same observers on 12 patients. Interobserver agreement was found to be 80% or better in all categories when exact correspondence was required. Agreement was better than 94% when one-step difference was allowed. Intraobserver agreement was 94.8% and 94.7% for the two observers, calculated on the basis of the 544 observations possible in each patient. Detailed statistics used to demonstrate point to point inter- and intraobserver correspondence will be discussed.

The total scores in a group of 12 rheumatoid hands examined in duplicate ranged from one to 76. The difference between left and right hand scores in each patient ranged between 0 and 22, with a mean difference of 7 for each observer, calculated separately. The mean difference between duplicate total scores was 0 ± 7.5 and 0 ± 7.5. The value of the method was substantiated by scores in 3 patients treated with multiple intra-articular corticosteroid injections in one hand one year before evaluation. The treated hands had scores of 16, 47 and 5 while the untreated hands had scores of 42, 129 and 27, respectively.
A Prospective Study of the Rheumatoid Biologically Active Factor (RBAF): The Second Assessment

MURRAY B. UROWITZ, DUNCAN A. GORDON and IRVIN BRODER, Toronto, Canada

The serum and synovial fluid of patients with rheumatoid arthritis commonly contain a factor, the RBAF, which resembles a soluble antigen-antibody complex in its biologic, biochemical, and immunologic properties (Clin Exp Immunol 3:55, 1968). A prospective clinical and laboratory study in 127 patients with definite or classic rheumatoid arthritis revealed that the RBAF correlated with a more advanced and widespread form of disease (Clin Exp Immun 5:57, 1969).

The present report is concerned with the results obtained in a second assessment completed in 107 of the patients originally studied. The mean interval between the first and second assessments was 9 months. The overall prevalence of the RBAF in serum was 27%, similar to that found in the first assessment. However, the RBAF status had changed from negative to positive in 11 patients and from positive to negative in 13. The group who converted to positive had become clinically worse than the group who converted to negative in a number of the parameters assessed.

| Serum RBAF | Grip strength | Synovial Lansbury | Systemic index | Steroids |
|------------|---------------|-------------------|----------------|---------|
| — to +     | 107 mmHg      | 91%               | 58             | 64%     |
| + to —     | 155 mmHg      | 46%               | 33             | 15%     |

These findings could not be explained on the basis of differences in disease duration or length of interval between assessments. Therefore, the results of this study would seem to indicate that a change in RBAF status is associated with a corresponding change in the severity of rheumatoid arthritis.

The Effect of Psychosocial Factors on Rehabilitation in Chronic Rheumatoid Arthritis

PAUL J. VIGNOS, JR., HELEN M. THOMPSON, STEPHEN L. FINK, ROLAND W. MOSKOWITZ, KATHRYN H. SVEC and SIDNEY KATZ, Cleveland, Ohio

Previous studies of rehabilitation of rheumatoid arthritis patients have centered on medical aspects rather than psychosocial and intellectual factors. Therefore, in a controlled 1-year study of comprehensive care in active chronic rheumatoid arthritis, the interrelationships of intensity of care, psychosocial factors and functional abilities were evaluated. Stage 2 to 4 (ARA) ambulatory patients were randomly assigned to an "intensive" treatment group which received coordinated multidisciplinary clinic and home care, and control patients who received "routine" clinic care. Initially, both groups were comparable with respect to disease activity, social adjustment, functional ADL performance and intelligence scores. A high intellectual score was significantly related to fewer deteriorations in ADL than a low intellectual score. Most patients had poor social adjustment initially, but after 1 year of "intensive" treatment, three quarters of the patients in the higher intelligence group were classified as well adjusted socially. Among patients who maintained their ADL functional level, a larger number improved in social adjustment. More well motivated patients in the "intensive" care group maintained their ADL function than did poorly motivated patients in either the "intensive" care or control groups. Improvement in disease activity, as measured by Lansbury Index, proved to be independent of intelligence level, social adjustment or motivation scores, but was related to participation in the "intensive" treatment program. These findings suggest that controlled rehabilitation studies should take into consideration nonmedical factors which may have a significant bearing on therapeutic results.

Coexistence of Gout and Arterial Thrombosis

F. J. VIOZZI, G. B. BLUHM, J. M. RIDDLE and M. I. BARNHART, Detroit, Michigan

We have previously reported electron microscopic evidence that platelets from patients with primary gout exhibit increased surface spreading and aggregation. The ex vivo addition of uric acid, but not oxypurines, enhanced activation of normal platelets. Because of these findings, we initiated a
clinical survey to determine the prevalence of arterial thrombosis in a group of 280 patients diagnosed as having primary gout. The frequency distribution of these patients by the fourth to the ninth decades was as follows: 28, 63, 94, 66, 21 and 2. Ages ranged from 30 to 89 years, and only 3 were females. Fifty-one (18%) of these patients experienced an arterial thrombosis. Twenty-two (43%) were diagnosed as myocardial infarction (MI), 20 (39%) as cerebrovascular occlusive disease and 9 (18%) as peripheral vascular occlusive disease. Reports in foreign publications suggest (in certain cases) the coexistence of hyperuricemia and occlusive vascular disease. Published US population studies suggest a prevalence of arteriosclerotic heart disease including MI in the various decades as follows: 0.5% for the fourth, 0.9% for the fifth, and 6.0% for the sixth. In contrast, MI occurred in our gout patients in the following manner: 2 of 28 (7.1%) in the fourth decade, 4 of 63 (6.3%) in the fifth decade and 7 of 94 (7.4%) in the sixth decade. Thus, in the fourth and fifth decades, we found an increased incidence of arterial thrombosis in our patients with gout when compared with the general population. Primary gout appears to predispose to arterial thrombosis perhaps by accelerated platelet participation by either a direct urate-membrane action or production of altered vessels.

**Arthritis Due To Airborne Fluorides**

G. L. WALDBOTT, Detroit, Michigan

Fluoride compounds are common air pollutants emitted from numerous manufacturing processes. Fluoride enters vegetables and fruit through contaminated air and soil. Food of animal origin contains excess fluoride when domestic animals consume fluoride-polluted forage.

Arthritis especially in the spine and small finger joints featured the illness of 33 individuals residing within one-third to 3 miles of three fluoride-emitting factories. Eighteen manifested evidence of gastritis and enteritis; 22 of headaches and such other neurologic features as muscular fibrillation, spastic reflexes, incipient retinitis; and features of chronic fluoride intoxication in the preskeletal stage. Fluoride levels of ingested food exceeded those from nonpolluted areas by as much as 20 times.

The diagnostic features of the disease and laboratory data, including fluoride assays of bones, soft tissue organs and urine are presented.

**Two Distinct Responses to Prolonged Estrogen Administration in NZB/NZW Female Mice: Evaluation of Thymic Morphology and Serum \( \gamma \) Globulin Levels**

SARA ELLEN WALKER and GILES G. BOLE, Ann Arbor, Michigan

We have described two patterns of serum \( \gamma \) globulin (GG) response to 17-\( \beta \)-estradiol (2.5 \( \mu g/kg/day \)) in female NZB/NZW mice. After 6 weeks of treatment, Group 1 females responded with GG 9.7 \( \pm \) 2.4 and Group 2 females had no elevation of GG (4.9 \( \pm \) 1.4 \( \mu g/ml \)). Seroologic abnormalities (ANA, cryoproteins, \( \pm \) LE cell tests) were 3 times more common in Group 2 mice. Four-week-old NZB/NZW female mice were treated with 17-\( \beta \)-estradiol, and littermate controls received propylene glycol vehicle. Animals were killed after 4, 8, 12, 16 and 20 weeks of treatment. Serum protein electrophoresis and tests for serologic abnormalities were done. Mitotic figures in six 50\(^2\) areas of thymic cortex were counted, and the thymic cortex:medulla ratio (C:M ratio) was measured. Renal histology was evaluated. Unexpectedly, 5/10 Group 1 and 4/13 Group 2 animals died during treatment. Surviving mice maintained 2 distinct responses to estrogen. Group 1 had a cortical mitotic count of 4.8 \( \pm \) 0.8 after 8 weeks and a C:M ratio of 0.92 after 12 weeks. Group 2 mice had a mitotic count of 8.9 \( \pm \) 1.4 at 8 weeks and a C:M ratio of 3.89 \( \pm \) 1.63 at 12 weeks. Control mitotic count was 2.3 \( \pm \) 0.5 at 8 weeks, and control C:M ratio was 2.78 \( \pm \) 1.26 at 12 weeks. Group 1 mice maintained high levels of GG through 12 weeks of treatment (11.6 \( \mu g/ml \)). After 16 weeks, one Group 1 mouse showed a fall of GG to 4.9 \( \mu g/ml \) and became ANA+. A sustained rise of GG levels to 15.3 \( \pm \) 4.6 \( \mu g/ml \) occurred in Group 2. In control mice, GG rose to 8.8 \( \pm \) 2.8 \( \mu g/ml \) after 20 weeks. Between 6 and 20 weeks only one Group 1 animal became ANA+, and cryoproteins were found in the serum of a single Group 2 mouse. Five control animals developed serologic abnormalities. All mice had glomerular lesions. Long-term treatment with small doses of a naturally
occurring estrogen maintains 2 serum GG and some serologic abnormalities and shortens the life span of NZB/NZW female mice.

Membranolytic Effect of Monosodium Urate (MSU) Compared with Calcium Pyrophosphate-Dihydrate (CPPD)

W. R. Wallingford and D. J. McCarty, Chicago, Illinois

Intraleukocytic MSU crystals from gouty joint fluid are usually not within phagosomes, whereas the reverse is true for CPPD crystals in pseudogout. A possible analogy to the reported phagosome lysis in monocytes after phagocytosis of silica (S) crystals and the lytic (HL) effect of S on erythrocyte membranes prompted this study.

The HL effect of MSU and CPPD on human erythrocyte (RBC) membranes was compared and reported inhibitors of S induced HL were studied. A 1.5% suspension of 3X washed RBC's in Hank's, pH 6.9, was incubated with 10 mg/ml of particulate at 37°C X 14 hr. Percent of complete lysis ± SD were: control 3.3 ± 0.22, S 96.6 ± 3.59, MSU 25.4 ± 3.1, CPPD 5.68 ± 0.25, starch (ST) 3.81 ± .34. A strong hydrogen bonding material, 0.5% polyvinylpyridine N oxide, blocked the membranolytic effect: control 1.86 ± 0.25, S 3.35 ± 0.42, MSU 6.55 ± 0.32, CPPD 4.7 ± 0.48, ST 3.81 ± 0.77. Plasma also markedly inhibited the crystal-membrane interaction. Solutions of MSU caused no HL. A time course experiment showed that Δ %HL/Δt was: S (2.5 mg/ml) 13.1% HL/hr, MSU 3.0, CPPD 0.31, and control .055. Changes in pH over the range 6.6 to 7.5 did not influence the crystal-membrane interaction. OD (260-Gilford) remaining in the supernate after absorption of PVPNO (4 mls 0.005% w/v at pH 6.8) with particles (25 mg/ml) was:

control 2.0085 ± .0361, S 0.8570 ± .2404, MSU 1.3100 ± .1060 and CPPD 2.0725 ± .0408.

These data show a striking difference in the effect of MSU and CPPD crystals on biomembranes. It is postulated that phagocytosed urate behaves like phagocytosed silica—ie, after digestion of coating opsonins, lysis of the phagosome occurs, leaving the particle free in the cytoplasm and the phagocyte dying of “gastric rupture.”

A C'5 Cleaving Enzyme and Leukotactic Factors in Rheumatoid and Nonrheumatoid Synovial Fluids

Peter A. Ward and Nathan J. Zvaifler, Washington, DC

Polymorphonuclear leukocytes are thought to play an important role in joint inflammation and destruction, but the factors responsible for their accumulation in the articular cavity are unknown. Synovial fluids from patients with rheumatoid arthritis and other inflammatory joint diseases have been studied for the presence of factors chemotactic in vitro for rabbit neutrophil granulocytes. Seventy percent of rheumatoid fluids contained complement derived chemotactic factors, consisting of a macromolecular complex C567, and/or a factor of low molecular weight. On the basis of studies using specific antibody, ultracentrifugation and gel filtration this latter factor (C'5a) has been identified as a cleavage product of the 5th component of human complement. In half of the rheumatoid fluids, there also exists an enzyme capable of producing a chemotactically active small molecular weight cleavage product (C'5a) when incubated with isolated human C'5 but not C'3. This enzyme is active at a neutral pH and can be suppressed by soybean trypsin inhibitor and e-amino caproic acid, and by esters bearing basic but not aromatic amino acids. It has a molecular weight close to bovine albumin. A similar, if not identical, enzyme can be extracted from lysosomal granules of leukocytes obtained from rheumatoid synovial fluid exudates and lysosomal granules of rabbit neutrophils. In inflammatory nonrheumatoid joint effusions, chemotactic activity is present in half of the fluids tested, and C'5 cleaving enzyme is present in one-third of such fluids. No enzyme or preformed factors have been found in synovial fluids from patients with osteoarthritis (5 cases) or systemic lupus erythematosus (4 cases). The presence in synovial fluids of chemotactic factors and a generator of chemotactic activity may be related to the pathogenesis of joint inflammation.
Low Friction Arthroplasty of the Hip in Rheumatoid Arthritis and Ankylosing Spondylitis

RICHARD B. WELCH and JOHN CHARNLEY, Near Wigan, England

A study of 307 low-friction arthroplasties (total hip replacements) done between the years 1962 and 1967 in patients with either classic or definite rheumatoid arthritis has been carried out. Their status at the time of surgical intervention was one of functional class and anatomical stage 3 or 4.

Absolute relief of pain has been afforded by this procedure in 94.7% of hips, and another 3.9% had only intermittent discomfort which disappeared quickly. Increase in passive range of motion has been equally as impressive. The rheumatoid patient's existence within a limited geographic area has been greatly aided because they have obtained pain relief and increase in motion. Their ability to accomplish distances has been augmented, but not to any great degree due to their overall involvement.

Arthroplasties that have been recorded as total failures are limited to those that have become infected or are a result of a technical error. The problems and complications encountered in carrying out these procedures are to be discussed.

In Vitro Studies of Possible Cell-Mediated Tissue Injury in Rheumatoid Arthritis

EDWARD WHITE and J. B. PETER, Los Angeles, California

Sensitized lymphocytes can be stimulated with the appropriate antigen to produce cytotoxic factors. These cytotoxic factors may be important in tissue injury. Supernatants or ultrafiltrates from cultures of lymphocytes stimulated either nonspecifically with phytohemagglutinin or specifically with the appropriate antigens produce cytopathic effects on mouse fibroblast monolayers. These effects can be measured qualitatively by microscopic observation or quantitatively by assay of protein synthesis in the monolayers. Using this system, we found that supernatants from cultures of lymphocytes obtained from PPD positive patients incubated with PPD-killed fibroblasts in 4 out of 6 experiments, whereas similar studies of lymphocytes from PPD negative patients produced no cytotoxicity. PHA stimulation of normal human lymphocytes consistently resulted in production of soluble cytotoxins.

Lymphocytes from 5 patients with rheumatoid arthritis were cultured with (1) homogenates from rheumatoid synovium or (2) 500 g or 105,000 g supernatants of the homogenates. Ultrafiltrates from these lymphocyte cultures placed on mouse fibroblast monolayers produced no consistent cytopathic effects compared with controls. Synovial tissue from 3 patients with rheumatoid arthritis were used.

In other experiments, lymphocytes from 16 rheumatoid and 7 normal patients placed directly on monolayers grown from rheumatoid or osteoarthritic synovium showed varying degrees of cytotoxicity. Rheumatoid lymphocytes did not consistently show greater cytopathic effects. The variable degrees of cytotoxicity were possibly due to histoincompatibility.

Cytotoxin production by sensitized lymphocytes may be important in the tissue injury of rheumatoid arthritis, but our studies to date have provided no clear-cut support for this possibility.

Early Recognition of Radiographic Erosions in Rheumatoid Arthritis

ALAN H. WILDE, ALLEN MACKENZIE and WILLIAM PURVAN, Cleveland, Ohio

The early recognition of erosions of the subchondral bone of articular surfaces has significance with regards to the course and prognosis of rheumatoid arthritis. Bywaters has pointed out that any break in the continuity of the subchondral plate of bone should be suspected as representing an erosion of bone. We have studied the roentgenograms of 20 knee joints and compared them with photographs of the knee joint taken at the time of synovectomy. We have found that areas of indistinctness of the subchondral plate or actual loss of a portion of the subchondral plate are significant and do represent erosions of bone. There is also occasionally seen an area of radiolucency beneath an apparently intact subchondral plate. This radiolucent line may also represent an erosion of bone.
Composite Arthroplasty for the Arthritic Knee

PAUL YOUNG and STANLEY S. ATKINS, Asheville, North Carolina

Our team of orthopedic surgeon, rheumatologist, three physical therapists and two brace makers have been working together on reconstruction of the arthritic knee since 1964. For the first 4 years, we would select the surgery that the patient seemed to need. We would usually operate on the anterior compartment first. With this method, the position of the knee was satisfactory in only 25% of 53 knees after one operation. A second operation to the posterior knee or prolonged PT improved the results to good or excellent in 59% when evaluated 6 months after surgery.

During the last 2 years, we have been using the composite arthroplasty in which the same operation was used in every case. Incisions were made on the medial and lateral aspect of the knee. These are shifted anteriorly or posteriorly to permit surgery to both the anterior and posterior compartments at a single sitting. The posterior synovectomy, capsulotomy, tensor facia lata release and tendon lengthening are done in the posterior compartment first. Then the anterior synovectomy, remodeling of the articular surfaces and insertion of medial and lateral tibial plateau prostheses are done. With the composite arthroplasty, the position of the knee after one operation has been satisfactory in 100% of 25 knees. Results 6 months after surgery have been good or excellent in 95%.

While the patient is in the hospital, all steroids—indomethacin, phenylbutazone, etc—are stopped. The 35 patients with rheumatoid arthritis were managed with only gold, antimalarials and/or cyclophosphamide plus ad lib aspirin. The 11 patients with osteoarthritis received no medication. Long-term results on 36 knees evaluated 3 years after surgery showed that 83% were better than they had been 1 year after surgery. None had regressed. There was only one recurrence of synovitis in 27 rheumatoid knees.

Effect of Oxonic Acid, A Uricase Inhibitor, on Plasma and Urinary Uric Acid and Allantoin in the Mongrel and Dalmatian Dog

TS’AI-FAN YU, LAWRENCE BERGER, CLARA KAUNG and ALEXANDER B. GUTMAN, New York, New York

Oxonic acid (2,4 dihydroxy-6-carboxyl-1,3,5 triazine) is a potent competitive inhibitor of uricase in vitro (Fridovich J: Biol Chem 240:2491, 1965), and in vivo in the rat (Johnson et al: Fed Proc 27:403, 1968). In the present study in dogs, oxonic acid and uric acid in plasma and urine were separated by anion exchange chromatography; after elution from the columns, the respective UV absorption spectra were recorded for quantification. A modified Young’s method was used to estimate allantoin in plasma and urine.

In 7 experiments using mongrel dogs, plasma urate was first raised to 1.1—11.8 mg%, and urinary uric acid excretion to 0.39—6.84 mg/min by varying rates of uric acid in fusion. When oxonic acid was then infused (16—40 mg/min), plasma urate invariably rose further, to 3.7—21.2 mg%, and renal excretion of uric acid consistently increased, to 1.12—14.07 mg/min. Allantoin simultaneously decreased in plasma and urine. In contrast, in 9 experiments using dalmatian dogs, there was no detectable change in the plasma and urinary allantoin levels; nor did the plasma urate rise appreciably after oxonic acid infusion. Urinary uric acid excretion fell from 0.37—15.97 mg/min to 0.24—12.13 mg/min, and the mean C_{urate}/C_{in} decreased from 1.41 to 1.25.

Oxonic acid evidently inhibited uricase activity in the mongrel dogs, resulting in plasma and urinary uric acid and allantoin levels like those in untreated dalmatians. The dalmatian also possesses hepatic uricase, which however, is much less efficient in oxidizing uric acid to allantoin (as we previously reported), apparently because the enzyme is less accessible to uric acid substrate; it is also impervious in vivo to oxonic acid. The decrease in C_{urate} is ascribed to competition for the tubular organic acid system with oxonic acid, which was shown to be eliminated by tubular secretion.
The routine chest roentgenogram may lead to the diagnosis of rheumatoid arthritis (RA) by careful scrutiny of joints frequently visualized on the film, namely the glenohumeral (GH), acromioclavicular (AC), sternoclavicular, wrist and finger joints. The finding of abnormalities in the AC joint in some RA patients suggested a more detailed evaluation of this articulation.

Over 100 patients with RA were studied. Approximately 30% had abnormalities in the AC joint; these varied from small cystic areas erosions, and irregularity of the contiguous joint surfaces to advanced destructive changes with "tapering" or "penciling" at the end of the clavicle. Six patients had marked alterations in the AC joint (stage 3 or 4); similar advanced involvement was present in other joints. Of interest in these patients was the lack of correlation with the latex titer for rheumatoid factor: 3 had negative titers, 1 a titer of 1:320, and another 1:5120.

Five RA patients had GH joint changes without involvement of the AC joint; in 4, the degree of GH change was severe, graded 3 or 4+. Fourteen patients had both AC and GH abnormalities. There was no apparent correlation in the findings of these 2 articulations.

Control patients included those without arthritis, patients with osteoarthritis, rheumatoid spondylitis, other connective tissue diseases and gout. Except for gout, occasional minor lesions were found in the controls, but significantly less frequently. In gout, however, tapering and changes difficult to differentiate from RA were found in 2 of 19 patients.