cient acylation medium for the objects dried by water–alcohol steps or by freeze-drying. The time required for acylation is 1 to 3 days. The specimen is then washed in monomer and embedded as usual. Other acid anhydrides may be used, but acid chlorides are not recommended. The amides and ammonium salts produced by the foregoing acylation are indifferent to the vinyl polymerization and allow the resin to cure to full extent.

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## Binding of an Alpha Globulin to Hyaluronateprotein in Pathological Synovial Fluids

Abstract. *Rabbits were immunized with hyaluronateprotein isolated from normal or pathological synovial fluids. Immunoelectrophoresis of hyaluronidase-digested pathological hyaluronateprotein and its antiserum showed a single precipitin arc extending through both alpha globulin zones. Digested normal hyaluronateprotein formed no lines with either its antiserum or with that to pathological hyaluronateprotein. The antigenic component present in pathological hyaluronateprotein formed a reaction of complete fusion with a serum protein which was identified by immunoelectrophoresis as an alpha globulin.*

Ogston and Stanier (*1*) isolated hyaluronate containing 25 percent protein from bovine synovial fluid. Using quantitative precipitin techniques, Curtain (*2*) identified all bovine serum protein fractions except albumin in the hyaluronateprotein "complex."

When normal human synovial fluids were adsorbed with equal parts of IRC-50 resin and hydroxylapatite, and then repeatedly filtered through 0.1$\mu$ Millipore filters, the final product retained on the filter was hyaluronate firmly combined with 2 percent protein. We have called this compound normal hyaluronateprotein (NHP) (*3*). When synovial effusions from actively involved joints of patients with rheumatoid arthritis were similarly treated, the product isolated differed from that of normal fluid. Hyaluronateprotein from rheumatoid patients (RHP) contained more bound protein (about 10 percent) and, at *p*H 4.5, showed two unusual properties: gel formation, and failure to move toward the anode during zone electrophoresis (*4*). The possibility of an immunologic difference between NHP and RHP led to this study.

Rabbits were immunized with about 1 mg of either NHP or RHP emulsified in Freund's complete adjuvant. Booster injections without adjuvant were given 1 month and 6 months later.

When RHP (0.5 mg/g) was studied with antibody to RHP by agar double diffusion or by immunoelectrophoresis at *p*H 8.6, no precipitin lines formed. After RHP was digested with testicular hyaluronidase (150 turbidity reducing units, 30 minutes at 37°C), a single precipitin line was obtained with antibody to RHP on agar double diffusion (Fig. 1); immunoelectrophoresis showed only one precipitin arc extending through both $\alpha$-globulin zones (Fig. 2). Digestion with hyaluronidase was necessary for the RHP to diffuse through agar, but the immunologic reactivity of RHP with antibody to RHP did not depend on digestion with hyaluronidase; after a single absorption of antibody to RHP (1 ml) with whole RHP (2 mg), the intensity of the precipitin reaction obtained with digested RHP on agar double diffusion was markedly reduced. Hyaluronidase-digested RHP did not form a line with antibody to NHP. Digested NHP (0.5 to 1.25 mg/g) formed no precipitin arcs with either antibody to NHP or antibody to RHP. Antibody to RHP (1 ml) absorbed with NHP (10 mg) still formed a precipitin line with digested RHP, indicating that RHP contains an antigenic component not present in NHP. This antigenic component appears to be closely related to the protein moiety, for after tryptic digestion of RHP no precipitin line was obtained with antibody to RHP.

It was previously reported (*4*) that the higher protein content and unusual physical properties of RHP were not specific for rheumatoid arthritis, but were also found in hyaluronateprotein from gouty synovial fluids. Accordingly,

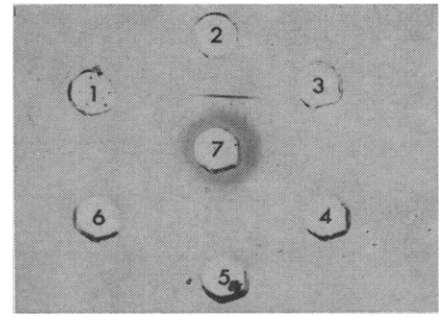


Fig. 1. Agar double diffusion of hyaluronateprotein from a rheumatoid patient (RHP) and hyaluronateprotein from a normal person (NHP) with anti-RHP. *1*, RHP; *2*, RHP digested with hyaluronidase; *3*, NHP; *4*, NHP digested with hyaluronidase; *5*, NHP isolated again after addition of rheumatoid serum, before hyaluronidase digestion; *6*, same as *5*, but after digestion; *7*, antibody to RHP.

the specificity of the immunologic reaction between antibody to RHP and RHP was investigated. Synovial fluids were obtained from affected knee joints of patients with various pathological conditions: gout (2 cases), rheumatic fever (1 case), psoriasis with arthritis (1 case), and synovitis of undetermined etiology (3 cases). Hyaluronateprotein isolated from these fluids, and digested with hyaluronidase, formed a single precipitin line with antibody to RHP on immunoelectrophoresis. On agar double diffusion with antibody to RHP in the center well, these digested pathological hyaluronateproteins (PHP) formed a single precipitin line that fused with

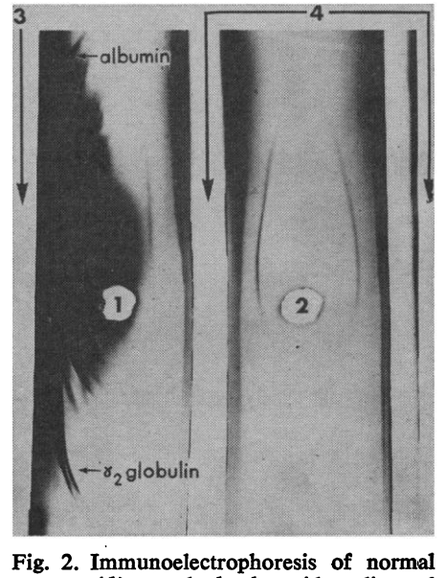


Fig. 2. Immunoelectrophoresis of normal serum (*1*), and hyaluronidase-digested RHP (*2*), with antibody to human serum (*3*), and antibody to RHP (*4*). Anode at top.

the line produced by digested RHP. The remainder of this paper is devoted to description of the reactions of hyaluronidase-digested RHP with antibody to RHP or antibody to human serum; it should be noted that similar results were obtained with hyaluronidase-digested PHP.

When hyaluronidase-digested RHP was studied with a potent antibody to human serum, a single precipitin line was obtained; this made it particularly important to show that the antigenic component was firmly bound in RHP and was not simply a serum protein retained as a contaminant. Evidence for the firm combination of hyaluronate and all the protein in RHP has been reported in detail (*4*); the major line of evidence is the identical electrophoretic mobility of hyaluronate and protein over a wide *p*H range (5.4 to 10.6). After zone electrophoresis at *p*H 7.4, RHP was isolated, digested with hyaluronidase, and studied by immunoelectrophoresis with antibody to RHP and antibody to human serum. A single precipitin line still appeared, indicating that the antigenic component was firmly combined to the hyaluronate. In an attempt to reproduce the conditions in pathological synovial fluids, 1 ml of NHP (3 mg/g) and 2 ml of serum from a patient with active rheumatoid arthritis were mixed and incubated at 37°C for 90 minutes. The NHP, after reisolation and subsequent digestion with hyaluronidase, showed no precipitin lines with antibody to RHP or antibody to human serum (Fig. 1). These results constitute strong evidence that the antigenic properties of RHP are not caused by a serum protein contaminant.

Since the antigenic component in RHP appeared to be related to a serum protein, the reaction of human serums with antibody to RHP was studied by immunoelectrophoresis. Normal as well as rheumatoid serums formed a single line identified on immunoelectrophoresis as an $\alpha$-globulin (Fig. 2). On agar double diffusion this line formed a reaction of complete fusion with hyaluronidase-digested RHP. Antibody to RHP absorbed with human serum no longer formed a precipitin line with digested RHP.

Thus RHP contains a protein immunologically closely related to, if not identical with, an $\alpha$-globulin in serum. Tests with specific antiserums to several $\alpha$-globulins show that this protein is not ceruloplasmin, haptoglobin, or $\alpha_2$-macroglobulin. The $\alpha$-globulin is present in normal synovial fluid but passes through the 0.1$\mu$ Millipore filter. Thus the $\alpha$-globulin is not combined with hyaluronate in normal synovial fluid, and it is not possible to combine NHP and the $\alpha$-globulin in vitro by adding rheumatoid serum to NHP. In the synovial membrane of pathological joints the condition of the hyaluronate may be altered so that it combines with the $\alpha$-globulin. The antigenicity of PHP described in this paper is due to the presence of this $\alpha$-globulin which accounts, at least in part, for the higher protein content of PHP (*4*). Although the unusual physical properties of PHP are dependent upon its higher protein content (*4*), the exact role of this $\alpha$-globulin in determining these interesting physical properties is not yet clear (*5*).

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## Radiation Action on DNA in Bacteria: Effect of Oxygen

Abstract. *In* Escherichia coli *cells ionizing radiation produces a degradation of DNA to approximately 50 percent of the total amount originally insoluble in trichloroacetic acid. There is also a reduction of synthesis. Oxygen inhibits the degradation process by a dose reduction factor of 4 and the synthesis process by a factor of approximately 1.5. Thus, at relatively low doses, radiation action on bacteria is very probably mediated through the DNA.*

One of the best-established modifiers of the action of ionizing radiation on living cells is dissolved oxygen. Gray *et al.* (*1*), Howard-Flanders (*2*), and others have reviewed this subject. The effect of oxygen on the uptake of phosphorus and sulfur in cells of *Escherichia coli* was observed by Pollard and Macaulay (*3*). Among the effects sensitive to ionizing radiation is the degradation of DNA. This was first shown in microorganisms by Stuy (*4*), and work on the degradation of DNA in *E. coli* has been reported by Miletic, Kucan, and Novak (*5, 6*). The fact that DNA synthesis continues after radiation at a reduced rate was shown by Pollard and Vogler (*7*) and independently by Billen (*8*).

Since any effect at all of radiation on DNA in the cell would be expected to be damaging, the question arises whether the "oxygen effect" is also apparent in degradation and in reduced synthesis of DNA. A strong oxygen effect has been found for degradation and a lesser effect for the reduction of synthesis.

The degradation of DNA can be very readily observed with a thymine-requiring mutant of *E. coli.* Such a thymine-requiring mutant can be given labeled thymine, and the subsequent fate of the label can be studied by determining whether the thymine is incorporated into large hydrogen-bonded material, which is precipitated by trichloroacetic acid (TCA), or whether it is released into the soluble fraction. By a combination of labeling of *E. coli* 15 T-L-, a mutant requiring both thymine and leucine for growth (*9*), with thymine, and of subjecting cells to radiation, the degradation of the DNA has been estimated directly by observing that part of the label which appears in the TCA insoluble fraction of the cell extract.

Also if the cells are treated with oxygen by bubbling the gas through the suspension and are then irradiated with $Co^{60}$ $\gamma$-rays, the effects of radiation are increased fourfold as compared with cells that had been similarly exposed to nitrogen. The dose-reduction factor is defined as the ratio of doses that produce the same observed effect, the factor being 4 in this case. In Fig. 1 we show the results of two experiments in which 10- and 20-kr doses were given. Here the cells were first grown on medium supplemented with $C^{14}$-thymine through several periods of division; just prior to the experiment the cells were "chased" with "cold" thymine for 15 minutes. In such cells the pool of thymine as measured in our laboratory corresponds to 10 minutes' growth time. Thus the 15-minute "chase" essentially eliminates the whole pool of thymine and leaves only labeled thymine in the DNA. The time