served with the previous preparation.

The results (Table 1) confirm those of the first experiment, since secondary 7*S*-A injected 2 days after immunization partially suppressed the primary 19*S* and 7*S* responses. The results also indicate that secondary 7*S* antibodies can differ markedly in their capacities to inhibit antibody formation, that 7*S* antibody injected at the time of immunization, rather than 2 to 3 days later, inhibits 19*S* formation more effectively and 7*S* formation less effectively, and that 19*S* antibody does not influence 7*S* formation but can depress 19*S* formation. The capacity of 19*S* antibody to inhibit 19*S* antibody formation may not occur during formation of antibody to ϕX, however, since inhibition was only demonstrated when antibody concentrations usually attained at 1 week were achieved through passive administration at the time of immunization.

It would appear, therefore, that 19*S* antibody is considerably less effective than 7*S* antibody in the inhibition of antibody formation to ϕX. This difference between antibodies may be related to their class of immunoglobulin or to the stage of immunization at which they were obtained or both. That primary 7*S* antibody obtained 2 weeks after immunization was effective as an inhibitor suggests that the class of immunoglobulin is an important factor, but a more definitive test of 19*S* antibody inhibitory capacity would necessitate the use of a different immunization system in which long-term 19*S* synthesis occurs.

There are several possible explanations for the relative inability of 19*S* as compared to 7*S* antibody to inhibit antibody formation, principally: (i) There may be decreased binding affinity for antigen. (ii) There may be a rapid loss of antibody activity. γ_{1M}-Globulin has a half-life in the circulation of only 24 hours (*4*); within reticuloendothelial cells, the half life may also be extremely short, a situation analogous to the rapid loss of 19*S* antibody activity in vitro after treatment with certain enzymes (*9*) and sulfhydryl agents (*10*). (iii) The localization of antibody may differ; intravenously administered 19*S* antibody may not have access to a site of antigen accumulation.

None of these possibilities can be excluded at this time.

The marked differences in inhibitory capacities among antiserums containing 7*S* antibody also suggest that significant inhibition of antibody formation only occurs after a sufficient amount of the proper antibody has been formed. These findings are consistent with the well-known observation that a secondary antibody response can usually be obtained in the presence of a high concentration of serum antibody, but that repeated immunizations become less effective in stimulating antibody formation (*11*). It is suggested therefore that antibody formation "feedback" is one line of immunological defense against hyperimmunization; evidence presented elsewhere (*12*) suggests that induction of immunological tolerance is another.

The site of interaction between inhibitory antibody and antigen that otherwise would have stimulated antibody formation is not known. The studies presented here suggest that the site is extracirculatory. In accordance with current concepts of the essential role of the reticuloendothelial system in "processing" antigen before immunization (*13*) and the demonstration of anatomical continuity between reticuloendothelial cells and lymphoid cells in immunized lymph nodes (*14*), we suggest that this site may be within reticuloendothelial cells. Thus, antibody γ-globulin which also enters these cells may interact with antigen before such antibody undergoes catabolism.

MARTIN S. FINKELSTEIN
JONATHAN W. UHR
Irvington House Institute and
Department of Medicine, New York University School of Medicine, New York

References and Notes

1. T. Smith, *J. Exptl. Med.* **11**, 241 (1909); A. T. Glenny and H. J. Sudmerson, *J. Hyg.* **20**, 176 (1921).
2. J. W. Uhr and J. B. Baumann, *J. Exptl. Med.* **113**, 935 (1961).
3. ———, *ibid.*, p. 959.
4. J. W. Uhr and M. S. Finkelstein, *ibid.* **117**, 457 (1963).
5. Obtained from Dr. R. Sinsheimer.
6. J. W. Uhr, M. S. Finkelstein, J. B. Baumann, *J. Exptl. Med.* **115**, 655 (1962).
7. Groups of two to six nonimmunized guinea pigs were injected with the various antiserums used in these experiments, and the antibody concentration of the serums was determined at 1 and 3 to 4 weeks. These values were subtracted from the concentration of antibody in those immunized animals that received antibody passively in order to determine the amount of antibody actively formed. 2-Mercaptoethanol was also used to determine the concentration of 7*S* antibody present; by subtraction from antibody concentrations of untreated serum, the concentration of 19*S* antibody was defined. There was excellent agreement between the two methods.
8. Both 7*S* γ_1 and γ_2 antibodies for ϕX were demonstrated in hyperimmune serum by starch-block electrophoresis performed for us by Dr. B. Benacerraf.
9. E. C. Franklin, personal communication.
10. D. C. Bauer and A. B. Stavitsky, *Proc. Natl. Acad. Sci. U.S.* **41**, 1667 (1961).
11. J. Sterzl, *Excerpta Med. Sect. IV* **9**, 595 (1956); G. Gross and S. Woessner, *Pathol. Microbiol.* **26**, 439 (1963); I. Gery, personal communication.
12. M. M. Dorner and J. W. Uhr, *J. Exptl. Med.* **120**, 435 (1964).
13. M. Fishman, *ibid.* **114**, 837 (1961).
14. M. D. Schoenberg, V. R. Mumaw, R. D. Moore, A. S. Weisberger, *Science* **143**, 964 (1964).
15. Research conducted in part under the sponsorship of the Commission on Immunization of the Armed Forces Epidemiological Board, and supported in part by the Office of the Surgeon General, Department of the Army; aided by USPHS grant AI-01821-07.

15 May 1964

Hampered Vinyl Polymerization by Embedding Biological Objects

Abstract. *Inhibition of vinyl polymerization by the embedding of biological specimens is due to the presence of biogenic amines. This inhibition is eliminated by acylation of the embedded objects with acid anhydrides.*

Acrylic ester formulations and styrenated unsaturated polyester resins are often used as embedding media for biological specimens (*1*).

Acrylics are also used in the preparation of ultramicrotome cuts and with usual microtome techniques in the preparation of cuts of hard objects like beetles (*2*).

In many cases one can observe zones, with different light refraction, around the object in the innermost part of the casting resin. Such zones tend to develop bubbles after several weeks or months. This behavior is especially well known with molluscs, fish, and isolated organs of higher animals, but practically no embedding process for animal specimens is without this drawback.

The formation of such zones of incomplete polymerization surrounding the embedded objects is due to inhibition, by biogenic amines, of the local vinyl polymerization. Primary and secondary amines (such as those from peptide side chains) interfere, but tertiary amines generally are unreactive in this way, or they accelerate the polymerization. Horner and Schwenk have considered the reactivity (as initiators) of peroxides with amines (*3*).

The inhibition by biogenic amines can be eliminated by acylation and salt formation by means of suitable acid anhydrides. A 10 percent solution of acetic anhydride in anhydrous acetone or anhydrous monomer is an effi-

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.

A. A. HOFER
E. LAUTENSCHLAGER
Mathematisch-Naturwissenschaftliches Gymnasium, Basel, Switzerland

References and Notes

1. A. J. Spilner, *Mod. Plastics* **31**, 129 (1953); E. Lautenschlager, in preparation; C. E. Wright, *Mod. Plastics* **29**, 113 (1951).
2. W. Rathmayer, *Experientia* **18**, 47 (1962).
3. L. Horner and E. Schwenk, *Angew. Chem.* **61**, 411 (1949); *Ann. Chem.* **566**, 69 (1950); *J. Polymer Sci.* **18**, 438 (1955).

10 July 1964

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 0.1μ 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 α-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,

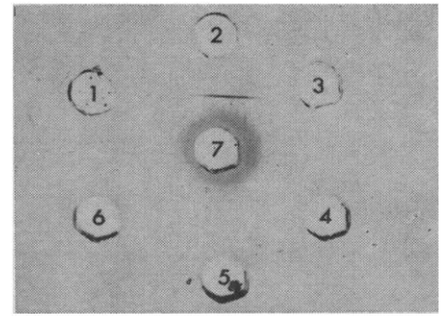

Fig. 1. Agar double diffusion of hyaluronateprotein from a rheumatoid patient (RHP) and hyaluronateprotein from a normal person (NHP) with anti-RHP. *1*, RHP; *2*, RHP digested with hyaluronidase; *3*, NHP; *4*, NHP digested with hyaluronidase; *5*, NHP isolated again after addition of rheumatoid serum,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

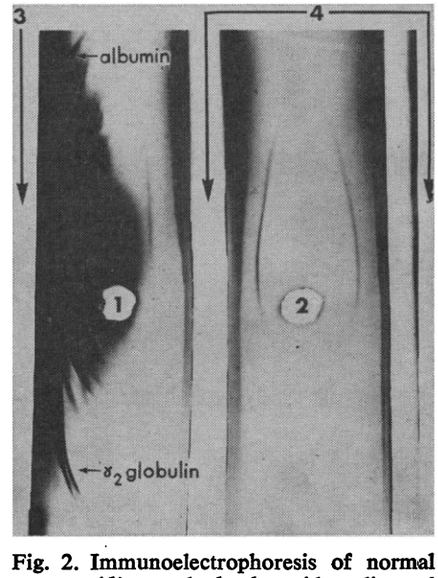

Fig. 2. Immunoelectrophoresis of normal serum (*1*), and hyaluronidase-digested RHP (*2*), with antibody to human serum (*3*), and antibody to RHP (*4*). Anode at top.