achieved, *i.e.*, from 15 to 100 units per ml. However, the isolating procedure is carried out at a net loss, *i.e.*, from 15000 units to 6000 units or a net recovery of about 40%. The value of the procedure derives from the increased concentration per unit volume.

Anticomplementary titers of the products were run in conjunction with the properdin assays and in each case the product was shown to be non-anticomplementary. The final product had the paper electrophoretic characteristics of gamma globulin.

The purification step achieved by inadvertent discovery of the insolubility of the properdin-containing fraction at 0°C was apparently made possible by the prior concentration steps. The possible relation of "cryogenic" proteins(8,9,10) to the partially purified properdin. which acts similarly to described cryogenic globulin, remains to be determined.

*Summary.* A procedure for isolation of properdin from bovine serum using cold ethanol fractionation technics is reported. Results indicate that high yields of properdin per unit volume are obtainable without use of zymosan.

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## Sensitized Sheep Cell Hemagglutination Reaction in Rats with Experimental Infection of Bone and Joint. (24312)

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A reproducible infection of bone and joint of rats has been produced in high incidence with a strain of *Streptobacillus moniliformis* isolated from a rat with middle-ear infection (1). Although this microorganism is considered of low virulence or a commensal for rats(2,3), this strain isolated in our laboratory has shown a specific affinity for joints, bone, and periarticular tissues after intravenous injection. To date, 109 out of 116 animals injected intravenously with this microorganism have developed gross and/or microscopic lesions. Of those animals showing acute changes, the infecting microorganism has been recovered from 19 of 21 wrists or ankles cultured during the acute, 5-11 day stage. Blood cultures made during this same 5-11 day stage have been positive in only 1 of 15 animals. The involvement of the region of the joint in the inflammatory process has directed immunologic studies of this infection into investigation of possible similarities to serologic reactions seen in human rheumatoid arthritis. Observations that the serum of patients with rheumatoid arthritis agglutinated sensitized bacteria, red cells, and other particles(4,5,6), have led to development of the sensitized sheep cell agglutination (SSCA) reaction as a phenomenon occurring most frequently in rheumatoid arthritis(7,8,9,10). This agglutination reaction has been found positive less frequently in some of the so-

TABLE I. Gross Changes in Rats following Intravenous Injection of *Streptobacillus moniliformis.*

| Exp. No. | No. rats infected | No. rats with lesions | Regions involved | | | | |
|---|---|---|---|---|---|---|---|
| | | | Left wrist | Right wrist | Left ankle | Right ankle | Other |
| 48 | 8 | 8 | 3 | 6 | 3 | 2 | |
| 50 | 35 | 33 | 22 | 27 | 12 | 11 | Left hip–1<br>Right hip–1 |
| 53 | 12 | 12 | 11 | 10 | 2 | 5 | Right hip–1 |
| Total: | 55 | 53 | 36 | 43 | 17 | 18 | 3 |

called "collagen diseases," such as disseminated lupus erythematosus, polyarteritis nodosa, scleroderma, and dermatomyositis(6,8, 9,11,12,13,14). Several modifications of the agglutination reaction have been proposed and reported as yielding significantly elevated titres in 44 to 95% of patients with rheumatoid arthritis(11,12,13,14,15).

The agglutination test applied in the current experiments was a slight modification of the euglobulin test devised by Ziff *et al.*(16). Briefly, this consists of testing a euglobulin fraction of the serum proteins obtained from serum by dialysis against a low ionic strength buffer at pH approximately 6.0. This procedure enables one to use a sensitizing dose of anti-sheep erythrocyte serum as great as one-half the basic agglutinating dose without materially increasing the false positive reactions. Studies employing euglobulin fractions of serum have been reported as yielding 86-91% positive reactions in human rheumatoid arthritis(16,17,18).

*Materials and methods.* A. Production of lesions. Technics previously described were employed to produce lesions of joint regions in rats(1). In brief, young Holtzmann-Fisher cross rats, 2½-3½ months old, raised free of infection, were injected intravenously (tail vein) with 2.0 ml of a 20-22 hour old broth culture of *S. moniliformis.* Cultural methods have been described. A total of 55 rats in 3 separate experiments were injected, and of these, 53 developed gross changes in wrists, ankles, and occasionally elsewhere. These were manifested by redness, swelling, or tenderness of one or more joint regions, appearing usually 5 to 7 days after injection. Incidence and distribution of lesions in infected animals is shown in Table I. Animals were killed with ether on the 6th or 7th day after injection, and 2 to 5 ml of blood were drawn from the inferior vena cava. A total of 53 uninfected rats were killed with ether and bled in similar fashion to serve as normal control animals. Some of the sera from these controls were run simultaneously with sera from infected animals (Exp. 52 and 53).

B. Modified sensitized sheep cell agglutination test. 1. *Cell suspensions.* Sheep blood was collected in sterile flasks containing 100 ml of an anticoagulant and preservative solution of 0.94% disodium salt of ethylene-diamine tetraacetate (EDTA) (adjusted to pH 6.0 with NaOH) and 5.0% glucose. The cells were washed in 0.85% NaCl solution and stored as packed cells in the anticoagulant solution at 5°C. When used in tests, the packed cells were washed in saline and made to 1.5% or 3.0% suspensions of cells in a solution of one part 0.03 M EDTA (adjusted to pH 7.0), ionic strength 0.168, and 9 parts 0.85% NaCl. 2. *Sensitized cells.* Commercially available anti-sheep erythrocyte rabbit serum (Amboceptor) in 50% glycerine solution was made to 1:100 dilution of serum in 0.85% NaCl containing 0.2% phenol preservative. This was again diluted for determination of the basic agglutination titre (B.A.T.)(21), and tested against a 1.5% cell suspension. Amboceptor solution at twice the BAT dilution was added to an equal volume of 3.0% cell suspension, held at least 30 minutes at room temperature, and then overnight in the refrigerator before use. This comprised the sensitized 1.5% erythrocyte suspension. 3. *Serum euglobulin.* Sera for test were diluted with an equal volume of distilled water and were dialyzed overnight against EDTA solution (pH 6.0, ionic strength 0.0126) at 2°C to precipitate the

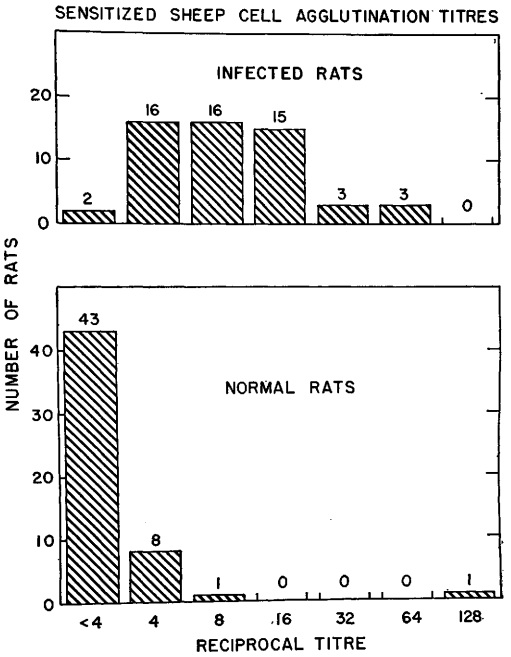

FIG. 1.

euglobulin fraction. Precipitates were washed twice in a volume of the same buffer equal to the serum volume used, and were redissolved in the pH 7 EDTA saline buffer. They were then inactivated at 56° for 30 minutes and absorbed at 37° according to Ziff(16), to remove Forsmann and other sheep cell agglutinins. 4. *Agglutination Titre.* Serial 2-fold dilutions, starting at a 1:4 dilution of rat serum euglobulin were made for the titration. 0.5 ml of 1.5% sensitized sheep erythrocyte suspension was added to 0.5 ml of each serum dilution. Tubes were held at 5°C overnight, and agglutination was read as follows. The tubes were inverted simultaneously 3 times in a covered rack, and the serum-red cell mixtures were allowed to settle for 1 hour. Agglutinations were graded from 0 to ++++, based on size and rate of settling of the red cell clumps. The highest dilution showing agglutination, *i.e.*, a definite agglutination pattern on the curved bottom of the tube, was taken as the agglutination titre(19).

*Results.* Serum euglobulin fractions of 55 rats infected with *S. moniliformis,* and of 53 normal uninfected rats were assayed for sensitized sheep cell agglutinins. The results of these tests and the distribution of titres developed by the 2 groups of rats is shown in Fig. 1. It will be observed that 43 of the 53 uninfected controls showed titres of less than 1:4, and 8 showed titres of 1:4. Only 2 rats developed higher titres, one of which was 1:128. In the infected group, only 2 rats showed a titre of less than 1:4, and 37 of the 55 developed titres greater than 1:8, a level which was exceeded by only 2 of the uninfected controls. This would appear to establish a titre of 1:8 as definitely abnormal, and indicative of response to this infection, under the conditions described. The possibility that the SSCA protein was not being recovered in the euglobulin fraction was excluded by testing the whole sera of uninfected rats. Of 27 such sera tested, only one had a titre of 1:4, indicating that the euglobulin method was definitely more sensitive for detection of low SSCA titres.

*Discussion.* An increase in sensitized sheep cell agglutinin titre was demonstrated in the euglobulin fraction of sera from rats which were experimentally infected with *S. moniliformis.* A high percentage of these infected animals developed lesions in or about joints within 5-7 days after injection of the microorganism, at which time sera were taken for testing. Although the majority of infected rats showed elevated titres, no extremely high titres were developed, and the striking difference was in the extremely low titres observed in normal controls. The absence of development of extremely high SSCA titres may be ascribed to the general lack of immunologic responsiveness noted in the rat (20), to the early acute stage of the experimental disease at which time sera were taken, or to basic differences in the human and murine diseases.

The high titres observed in human rheumatoid arthritis are not usually found until the disease has been established for 6 months or more(7,8,13,21), and only occasionally has the SSCA titre been reported elevated in early rheumatoid arthritis(22,23). Since the inflammatory process in the joint regions in these rats was at the acute stage, the finding of elevated titres in their sera is of added interest. The one significantly elevated serum titre in an animal from the uninfected group

may conceivably have been due to accidental or spontaneous infection of this animal with *S. moniliformis*, since this microorganism is extremely common in rat colonies and considerable vigilance is required to eliminate it completely.

It will be of interest to determine whether the sensitized sheep cell agglutinins of the rat are gamma globulins with a sedimentation constant of approximately 19S, as in the human serum studies of Franklin *et al.*, and whether these tend to complex with 7S gamma globulins to form complexes of 22S or greater in the same manner as in human rheumatoid arthritis. Since the current series of rats have developed low titres, and Franklin *et al.*(24) found that only high titre sera showed demonstrable complexes of 22S or greater, it may be necessary to maintain chronic or recurrent infections in order to achieve high concentrations of the high molecular weight complexes in rat sera. On the other hand, it may be necessary to investigate this phenomenon in other species.

*Summary.* 1) Rats infected with a strain of *Streptobacillus moniliformis* developed an inflammatory reaction in joint regions in 109 of 116 animals. 2) Sensitized sheep cell agglutination titres of the serum euglobulin tested from 55 infected rats were significantly higher than those of 53 uninfected controls.

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## Observations on Mechanism and Prevention of Non-Specific Agglutination of Leukocytes.* (24313)

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Leukocytes have a striking tendency to agglutinate non-specifically. Thus, leukocytes obtained by sedimentation from normal whole blood and incubated with any normal un-

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