Selectivity of Heparin-Latex Method and Sensitized Sheep Cell Test for Rheumatoid Serums.* ((24020)

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Since the agglutinating property of serums from patients with rheumatoid arthritis was put to use for diagnostic purposes, this procedure has undergone many changes. The hemolytic streptococci used in the first method(1) were replaced by sensitized sheep erythrocytes(2-5), collodion particles(6), polystyrene globules(7), or bentonite(8). In the original method using polystyrene latex(7), particles of uniform diameter were suspended in Fraction II of human serum(9), then incubated and centrifuged. Agglutination of latex by rheumatoid serum was considered a positive reaction. Gofton *et al.*(10) reported recently that latex particles coated with 1% solution of certain polysaccharides, instead of Fraction II, are also agglutinated by serums from individuals with rheumatoid arthritis. In the present investigation, a modified method using 0.5% heparin as sensitizing agent for latex particles is compared with the standard sensitized sheep cell test(5).

Materials and methods. A polystyrene latex suspension, with globules measuring 0.81 μ diameter and containing 11.2% solids,† was diluted in ratio of 1:10 with distilled water and filtered through Whatman No. 40 filter paper. The borate buffer was prepared by dissolving 6.184 g of boric acid in distilled water, adding 8 ml of 1 N sodium hydroxide and making final volume 2000 ml. The pH was adjusted to 7.9 and 17 g sodium chloride was added. Heparin was purchased from commercial source.‡ Serums from patients with rheumatoid arthritis were obtained at Arthritis Clinic of Grace-New Haven Community Hospital and from private patients of one of the authors (G. de F.). Some samples were stored to 12 months prior to testing. Control serums, furnished by Grace-New Haven Community Hospital, came mostly from different departments. Twelve control samples were obtained from healthy personnel from Yale University School of Medicine. The agglutinating procedure was carried out in 9 progressive dilutions. The first tube contained a serum dilution of 1:20 (1 ml) and progressive 2-fold dilutions were prepared to the ratio of 1:5120. A control tube containing buffer only was included for each serum. To all tubes was then added 1 ml of heparin-latex suspension, prepared by mixing 9.8 ml of buffer, 0.1 ml of 1:10 latex suspension and 0.1 ml 0.5% heparin in buffer. The tubes were then shaken 5 minutes on mechanical shaker, incubated in water bath at 56°C 90 minutes, and, after cooling, centrifuged at 2500 rpm 5 minutes. In positive tests, the latex particles coagulated to fine, sharply defined granules with a slightly turbid or clear supernatant. In a few instances, a fern-like pattern of precipitate was seen on wall of tube. Amorphous, gelatinous masses were sometimes observed in tubes with control sera. They differed, however, distinctly from the sharp granulation in the positive tests. Agglutination appeared in great majority of tests between titers 1:20 and 1:320 and, with few exceptions, was present to highest dilution used, *e.g.* 1:5120. A few serums tested with higher dilutions showed endpoint to be in the 1:10000 to 1:20000 range of the titer. In triplicate determinations on the same serums, the initial agglutination titer differed one dilution only and the coagulum was present to highest titer used. Several tests were made using 1% heparin-latex and uncoated

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‡ Organon, Orange, N.J.

TABLE I. Results of 3 Agglutination Tests with Rheumatoid and Control Serums.

Diagnosis	No. of serums	0.5% heparin & latex Pos	0.5% heparin & latex Neg	Sensitized sheep cells Pos	Sensitized sheep cells Neg	No. of serums	Latex only Pos	Latex only Neg
Definite rheumatoid arthritis								
Stage 1	4	2	2	1	3			
2	8	6	2	3	5			
3	47	42	5	30	17			
Total	59	50	9	34	25	42	28	14
Possible rheumatoid arthritis	9	4	5		9			
Juvenile " "	2		2		2			
Psoriasis	3	2	1	2	1			
Spondylitis	5	1	4		5			
Lupus erythematosus	2	2		2		2	2	
Non-rheumatoid patients	78	2	76		78	11		11
Healthy subjects	12		12		12	12		12

latex particles (without heparin). The latex suspension for the latter test was prepared by mixing 9.9 ml of buffer and 0.1 ml of 1:10 latex suspension.

Results. On preliminary testing, the latex fixation test, using 1% heparin as coating agent(10) gave a high proportion of positive reactions with control sera. By decreasing the heparin concentration to 0.5%, these positive reactions could be eliminated and the test still retained a high sensitivity. The results obtained with this modified procedure are given below.

Serums from 59 patients with definite peripheral rheumatoid arthritis were tested with both sensitized sheep cell method and 0.5% heparin-latex procedure. These individuals satisfied the Amer. Rheumatism Asso. criteria for "definite rheumatoid arthritis" and were further subdivided into stages of the disease. Also included were 9 patients with "possible rheumatoid arthritis," 5 spondylitics, 2 with juvenile arthritis, and 3 with rheumatoid arthritis and psoriasis. Seventy-eight serums from non-rheumatoid patients and 12 samples from healthy subjects served as controls. A number of both rheumatoid and control serums was also tested with uncoated latex suspension (without heparin). The overall results are summarized in Table I.

Results with sensitized sheep cells are given in units corresponding to *last* dilution still showing positive reaction. In the heparin-latex test, the *first* dilution showing agglutination was selected, as the reaction was present in most cases up to the highest dilution used, *e.g.* 1:5120.

With one exception, the heparin latex test was positive in all samples which were also positive with sensitized sheep cells. In 8 cases, both heparin-latex and sensitized sheep cells were negative, and in 17 samples the latex-heparin test was positive, sensitized sheep cells negative. The high proportion of negative tests with sensitized sheep cells can be explained partly by the fact that many of these individuals were in clinical remission. Of the 2 control groups, the 12 sera of healthy subjects were negative with all tests. The 78 samples from the Serological Laboratory showed 76 negative and 2 positive tests with 0.5% heparin-latex and they were all negative with sensitized sheep cell test. Of 42 rheumatoid serums tested by latex alone, two-thirds were positive, a higher proportion of positives than reported by Singer and Plotz (7) or Gofton *et al.*(10). Table II gives the results of 22 rheumatoid arthritis serums treated with all 3 tests. With latex only, the agglutination started mostly in tubes with *higher dilutions* and was usually present to 1:5120 titer.

Discussion. The exact mechanism of the agglutination reaction is not known at present. There is an increasing body of evidence, however, suggesting that the polysaccharides either participate directly in the reaction or at least affect it. Treatment of synovial fluids from rheumatoid patients with hyaluronidase was reported to convert atypical hemagglutination patterns to typically positive ones(11). The results of Gofton *et al.*

TABLE II. Comparison of Titers Shown by 22 Individual Serums when Tested by the 3 Methods.

Sensitized sheep cells	0.5% heparin & latex*	Latex only*
256	20	640
128	20	640
256	20	640
Neg	160	160
512	20	320
Neg	80	160
512	20	20
512	160	320
128	20	320
128	320	640
128	20	20
256	160	160
256	80	640
256	40	80
128	20	1280
256	20	20
128	80	640
128	320	640
256	20	640
256	20	320
128	80	160
256	40	160

* First dilution showing agglutination.

(10) and the present findings indicate that certain polysaccharides in system with polystyrene particles of definite size can bring about, or at least enhance, agglutination of latex particles by rheumatoid serums. A high proportion of positive reactions observed with non-sensitized particles suggests that the effect of some of the "coating" agents may be catalyst-like in nature and that possibly systems can be developed which would agglutinate without external sensitizers.

Summary. Results of an agglutination test using polystyrene particles sensitized with 0.5% heparin are described. Sensitivity and selectivity of this system for rheumatoid serums compares favorably with those of the standard sensitized sheep cell test method. A high proportion of rheumatoid serums was found to give positive reactions with non-sensitized latex particles.

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1. Cecil, R. L., Nicholls, E. E., Stainsby, W. J., *Am. J. Path.*, 1930, v6, 619.

2. Waaler, E., *Acta Path. et Microbiol. Scand.*, 1940, v17, 172.

3. Rose, H. M., Ragan, C., Pearce, E., Lipman, M. O., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 1.

4. Heller, G., Jacobson, A. S., Kolodny, M. H., *ibid.*, 1949, v72, 316.

5. Boisvert, P. L., Brockett, J. B., and deForest, G. K., *Yale J. Biol. Med.*, 1956, v28, 622.

6. Vallis, A. D., *Am. J. Med. Sci.*, 1946, v212, 716.

7. Singer, J. M., Plotz, C. M., *Am. J. Med.*, 1956, v21, 888.

8. Bozicevich, J., Bunim, J. J., Freund, J., Ward, S. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 180.

9. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahnt, F. W., Lever, W. F., Liu, C. H., Mittleman, D., Mouton, R. F., Schmid, K., Uroma, E., *J. Am. Chem. Soc.*, 1950, v72, 465.

10. Gofton, J. P., Thomas, J. W., Robinson, H. S., *Can. Med. Assn. J.*, 1957, v77, 1098.

11. Krehl, W. A., Boisvert, P. L., de Forest, G. K., Mucci, M. B., *Yale J. Biol. Med.*, 1957, v30, 30.

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Effect of Adrenalectomy on Albumin-Palmitate-1-C^{14} Oxidation.* (24021)

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Studies have suggested that the adrenal glands play an important role in fat metabolism. The effect of adrenalectomy on ketosis (1-6) has been used as an indicator of the role of adrenals in fat catabolism but the results of such studies have been contradictory. Recent work (7,8) with isotopic tracers indi-

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