an inflammatory synovial fluid (*i.e.*, rheumatoid arthritis and gout) from a non-inflammatory synovial fluid (*i.e.*, degenerative joint disease). Chung *et al*(4) also found it impossible to differentiate degenerative joint disease synovial fluid from that of rheumatoid arthritis by lipid analysis, as did Bole(5) who felt that the contribution to the total lipid by pleocytosis in synovial fluid was minimal. This apparent lack of correlation between the white cell count and lipid concentration of synovial fluid is of some interest since Buchanan(12) and Marks, Gellhorn, and Kidson(13) have established that leukocytes can synthesize lipids. It may be that, as suggested by isotopic data in the chylous fluid(2), the synovial membrane routinely contributes significantly to local lipid content. This suggestion is also supported by electron micrographs taken in our laboratory of a variety of synovial membranes which not uncommonly demonstrate lipids in the synovial lining cells.

Summary. Total fatty acid analyses of synovial fluids from patients with rheumatoid arthritis, gout, and degenerative joint disease and matching sera were carried out by gas chromatography. The relative fatty acid composition of synovial fluid was similar to that in serum. Palmitic, oleic and linoleic acids constituted the major components and myristic, palmitoleic, stearic and arachidonic acids were the minor components. The synovial fluid fatty acid concentration was roughly one-half to one-third of that of the matching serum. However, correlation between the synovial fluid fatty acid concentration and its white cell count was poor and total fatty acid analyses were not helpful in differentiating an inflammatory synovial fluid from a non-inflammatory fluid.

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Formalinized Tanned Cell Hemagglutination Test for Demonstration Of Autoantibodies in Myasthenia Gravis.* (31408)

AIDA Y. DJANIAN, ERNEST WITEBSKY AND ERNST H. BEUTNER
Department of Bacteriology and Immunology, State University of New York at Buffalo, N. Y.

Several immunological methods have been employed to demonstrate muscle antibodies in the sera of patients suffering from myasthenia gravis. These include direct immunofluorescent (IF) staining(1,2,3), complement IF staining(1,2), indirect IF staining(2,4), complement fixation(2,6) and tanned cell agglutination, using fresh cells(5,7). The last technique appears to be the most sensitive in our hands. However, the preparation of fresh tanned cells coated with muscle antigen is somewhat cumbersome and the product is unstable. An improved method has now been devised utilizing more stable formalinized cells. One aim of this report is to compare the results obtained with fresh and

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formalinized tanned cells. The second objective is to study the possible concurrence of muscle autoantibodies with tissue antibodies such as thyroglobulin and nuclear autoantibodies.

Methods. Immunofluorescent staining, the complement fixation test, and the tanned cell hemagglutination test for muscle antibodies in humans were performed as described previously(2,8). Nuclear antibodies and thyroid antibodies were detected as described previously(8,9). The formalinized tanned cell hemagglutination test for myasthenia gravis is carried out in the following way(10,11).

Human erythrocytes of blood group 0 are washed 3 times with physiological saline solution, then packed by centrifuging for 15 minutes at 2000 rpm. A 1% suspension of these packed cells is made in borate succinate buffer, pH 7.5 and 0.75% NaCl. To this suspension neutral concentrated formalin (40%) is added gradually, with continuous agitation, until the concentration of formalin reaches 10%. (For instance, to 900 cc of a 1 % cell suspension a total of 100 cc of concentrated formalin is added, 10 cc at a time, and after each addition the mixture is shaken and allowed to stand from 5 to 10 minutes before another 10 cc of formalin is added in the same manner, until finally 100 cc of formalin has been used.) This procedure takes about 1 hour. The cells formalinized in this way are then left at 4°C overnight. The next day the same quantity of formalin (100 cc) is added in the same way but this time the formalinized cells are left at 4°C for 48 hours (or until the cells have settled). The supernatant is then discarded and an equal volume (1000 cc) of sterile buffered saline is added. After 2 days the clear supernatant is siphoned off and replaced with 1000 cc of fresh buffered saline solution; this washing procedure is repeated 3 times more at 2-day intervals for the purpose of removing residual formalin. After the last washing, a 4% suspension of cells is prepared in borate succinate buffer (.05 M succinic acid, 0.5 M sodium tetraborate, .17 M sodium chloride) solution for storage. Before use the cells were washed twice in phosphate buffered saline (.035 N phosphate, .115 N sodium chloride). Aliquots are taken from this suspension (the remainder is stored in the refrigerator) and equal amounts of tannic acid (diluted 1:2000) are added. This mixture is then shaken well and kept at room temperature for 30 minutes. Then the cell suspension is spun down at 1000 rpm for 4 to 5 minutes and sediment washed 3 times with buffered saline solution. The first washing is made with a 7.2 pH phosphate buffered saline solution and the second and third with saline solution of pH 6.4. The final 2% tanned blood cell suspension in pH 6.4 buffered saline is then ready for use.

The coating of the formalinized tanned cells is carried out in exactly the same way as that previously described for the fresh tanned cells. Equal volumes of muscle globulin solution, pH 6.4, and tanned cells are mixed. The average concentration of muscle protein solution employed for coating is 50 to 100 μg per 1 cc. This mixture is allowed to stand for 30 minutes at room temperature. Then the cells are washed with 10 cc of 1 % normal human serum diluted in buffered saline, pH 6.4, three times. A final stock solution of 1.5% tanned and coated cells is then prepared. This suspension is ready for use.

The interpretation of positive results should differ from those of the fresh tanned cell test. With the non-formalinized cells characteristic patterns of folding are observed with positive sera. The formalinized tanned cells do not yield such a pattern. The positive results are indicated by the formation of a uniform sheet of cells while a negative reaction appears as a round "button" or "donut" of cells.

Results and discussion. To compare the sensitivity of the tanned cell technique as performed with fresh cells on the one hand and with formalinized cells on the other hand, a group of sera from 68 patients suffering from myasthenia gravis were each tested by both methods. Of these 68 sera, the same 28 gave positive results with both formalinized and non-formalinized tanned cells; 40 were negative. Most sera even yielded the same titer, within one dilution, by both test methods; a few sera differed in their titer by as much as 2 dilutions. Since none of the sera tested yielded positive reactions by one or

TABLE I. Occurrence of Autoantibodies in Human Diseases.

	Hemagglutination						Immunofluorescence		
	Muscle			Thyroglobulin			Anti-nuclear factor		
Disease	No. of cases	No. pos	No. neg	No. of cases	No. pos	No. neg	No. of cases	No. pos	No. neg
Myasthenia gravis	72	30	42	70	12	58	69	21	48
Thyroiditis	41	0	41	41	30	11	38	5	33
SLE and RA†	33	0	33	33	2	31	33	20	13
Other diseases	25	1*	24	25	4	21	25	3	22

* One serum of a patient with chronic diarrhea but no clinical signs of myasthenia gravis yielded a strongly positive reaction. This case was encountered accidentally in a routine immunofluorescent screening test for nuclear antibodies involving hundreds of sera. Upon further clinical study of this patient, a thymoma was found which was removed by surgery and biopsy of his skeletal muscle showed lymphocytic infiltration. It will be interesting to follow the clinical course of this patient.

† SLE and RA = systemic lupus erythematosus and rheumatoid arthritis.

the other test method alone, the 2 techniques were deemed to be equally sensitive. The formalinized cells, however, offer a distinct advantage in that when tanned and coated, they may be kept in the refrigerator for 1 to 2 weeks or in the deep-freeze (rapidly frozen) for as long as 6 months. If frozen in small aliquots the quantity needed for testing may be thawed at room temperature as needed. With the aid of such a ready reagent it may be possible to test further the concurrence of muscle antibodies with other autoimmune responses, the relation of muscle, heart and thymus antibodies, the frequency and potency of muscle antibodies in different clinical manifestations of myasthenia gravis and other problems.

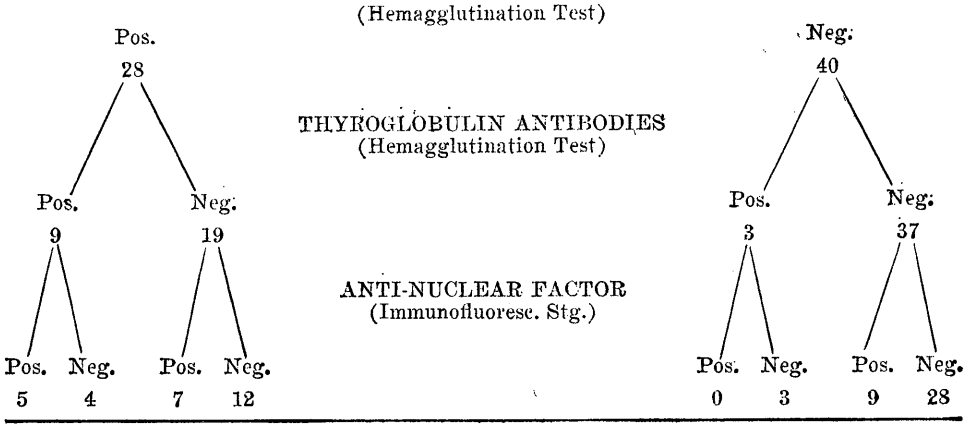
In view of the fact that a now readily available reagent was at hand for demonstration of muscle antibodies in myasthenia gravis, the tanned cell agglutination test using formalinized tanned cells was used to demonstrate their concurrence with other autoantibodies in human diseases. There are 3 human diseases in which autoantibodies have been demonstrated beyond any doubt in many laboratories and clinics, chronic thyroiditis (Hashimoto's disease), lupus erythematosus, and myasthenia gravis. It seemed of interest to examine this specificity in a number of patients' sera sent to our laboratory for diagnostic purposes.

As shown in Table I, the following conclusions can be drawn: in all 3 diseases under investigation, the largest percentage of positive reactions is obtained with the antigen directly related to the tissue involved; for instance, there are no antibodies against muscle tissue to be found in 41 sera from patients with chronic thyroiditis, though 30 of them contained thyroglobulin antibodies. Only 5 had antinuclear antibodies in this series. Conversely, of 33 sera from patients with systemic lupus erythematosus or rheumatoid arthritis, none had antibodies against muscle tissue; 2 had antibodies against thyroglobulin, but 20 showed antibodies against nuclear constituents. The situation regarding myasthenia gravis is somewhat different: while 30 out of 72 patients' sera contained antibodies against muscle tissue, 12 out of 70 contained thyroglobulin antibodies and 21 out of 69 revealed the presence of antinuclear factors.

To find out whether the simultaneous occurrence of thyroglobulin antibodies and antinuclear factors in the serum of patients suffering from myasthenia gravis was associated with circulating muscle antibodies, 68 of these sera were tested. The results are given in Table II. Of the 28 myasthenia gravis sera containing muscle antibody a greater percentage of these contained thyroglobulin antibody and antinuclear factor than the group of 40 myasthenia sera which did not contain muscle antibody. Whether this increase is as statistically significant as it appears to be on the basis of our findings requires further investigation.

Summary. A formalinized tanned cell agglutination test for detecting muscle antibodies in the serum of patients suffering from myasthenia gravis is described. The results obtained with the formalinized tanned cells

TABLE II. Results of Serological Examination of 68 Patients Clinically Diagnosed as Suffering from Myasthenia Gravis.



appear to be essentially the same as those obtained with fresh tanned cells. However, the formalinized and coated tanned cells can be kept in the refrigerator for 1 to 2 weeks or in a frozen state in the deep-freeze for as long as 6 months. Thyroid antibodies and antinuclear antibodies seem to occur more frequently in the sera of patients suffering from myasthenia gravis containing muscle autoantibodies than in groups of those patients without muscle antibodies.

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Measurement of Hemolysin Transfer Using Untreated Red Blood Cells. (31409)

HAZEL P. GUMP AND LAURENCE R. DRAPER (Introduced by Maurice Landy)
Laboratory of Physiology, National Cancer Institute, National Institutes of Health, USPHS, Bethesda, Md.

Taliaferro *et al*(1) determined the avidity of rabbit anti-sheep homolysin in terms of its intercellular transfer to which avidity is inversely related. They measured the proportion of hemolysin which transferred from a population of sensitized sheep erythrocytes