found that 11% of the strongly positive sera reacted with latex and buffer, without benefit of added globulin.

The reactivity of adult chicken serum with latex and buffer is without explanation at present. Perhaps the relative concentration of albumin to globulin in chicken serum or the pH or ionic influence of this particular buffer is of significance. Embryonic sera did not behave in this manner.

Summary. The sera from 7 animal species were found to contain globulin components which would function as "antigen" for rheumatoid sera in the latex-fixation test. The effectiveness of these sera as "antigens" depended on removal of albumin by either precipitation or dilution. Nine of 10 human sera behaved similarly. The addition of gelatin to gamma globulin resulted in loss of "antigenicity."

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Elution of Pollen Antigens from Tannic Acid Treated Erythrocytes.* (23396)

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The use of Boyden's technic of adsorbing protein antigens(1) onto tanned red cells allowed several investigators(2-6) to measure antibody levels against pollen antigens in hayfever serum. Orlans *et al.*(2) were able to detect antibodies in the sera of treated cases and of some untreated cases, but not in the sera of non-hayfever persons. Feinberg *et al.* (6) described similar findings, but also showed that the hemagglutinating antibody detected in these hayfever sera is not identical with the reagin type of antibody. All of the previously reported work used a multi-component antigenic extract of various pollens. The present study is concerned with a crude fractionation of the whole saline extract of short ragweed pollen to reduce the number of antigenic specificities, and with the fact that some antigenic components of short ragweed extracts slowly and spontaneously elute from the surface of tanned sheep erythrocytes.

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Materials and methods. Extracts of short ragweed pollen were Seitz filtered and stored as stock antigen at 10°C(6). The whole saline extract containing 116 μg protein N/ml was designated Antigen #1.

An aliquot of the whole short ragweed extract (10 ml) was treated with 4 ml of sterile, cold, 10% trichloroacetic acid (TCA) and allowed to stand at 10°C for one-half hour. The precipitate was removed, washed 3 times with cold 10% TCA, dissolved with pH 7.2 buffered saline, and diluted to the original antigen volume. This was designated Antigen #2.

The TCA non-precipitable supernate was

TABLE I. Hemagglutination Titration Immediately Following Preparation of RBC-Antigen Complex.

Antigen No.	No. hrs* incubation	Hemagglutination of tanned-S RBC-SR 20	40	80	160	320	640	1280	2560	C
1	2	4	4	4	4	4	4	3	2	—
	36	4	4	4	4	4	4	3	2	—
2	2	4	4	4	4	4	3	2	2	—
	36	4	4	3	1	—	—	—	—	—
3	2	4	4	4	4	4	4	4	3	—
	36	4	4	4	4	4	4	4	2	—
4	2	4	4	4	4	4	4	2	1	—
	36	4	4	2	—	—	—	—	—	—
5	2	—	—	—	—	—	—	—	—	—
	36	—	—	—	—	—	—	—	—	—
Control S RBC without antigen	2	—	—	—	—	—	—	—	—	—
	36	—	—	—	—	—	—	—	—	—

* After the 2 hr reading, the tubes were thoroughly mixed and re-incubated for 36 hr.

recovered and placed in a dialyzing sac, aseptically. This fraction was dialyzed against 4500 ml of 0.85% NaCl solution over a 3 hour period (1500 ml hr with constant stirring). The volume was then concentrated from 13 to 8 ml. The fluid was removed aseptically and was still TCA negative. This is Antigen #3.

Antigen #4 was prepared by digesting 2 ml of short ragweed Antigen #1 with 2 ml of twice crystallized trypsin (0.066 mg) at 37°C for 44 hrs. This solution, after the 44 hrs incubation, was inactive for digesting 1% gelatin. The digested short ragweed material was precipitated with 10% TCA, as above. The precipitate, dissolved and resuspended as above, was designated Antigen #4. The supernate, after dialysis, was concentrated and designated Antigen #5.

Rabbit anti-short ragweed serum, which contained precipitins to Antigen #1, was used in hemagglutination tests. The details of the technic are described elsewhere(6). Washed sheep RBC were treated with 1:20,000 tannic acid for 10 minutes at a pH of 7.2. The tanned cells were washed and exposed to the various antigen preparations at pH 6.4. The tanned cells with antigen adsorbed were stabilized with washes of normal rabbit serum (1:200). Titrations were performed with 2-fold serial dilutions of immune sera. Pattern readings were made after 2 hours at room temperature. The tubes were then thoroughly agitated and the tests reincubated for 36 hours, at which time the titers were again recorded.

Aliquots of the tanned sheep RBC, with the various antigen preparations adsorbed on the surface, were incubated in the cold for 36 hours. The cells were centrifuged and the supernatants were removed for skin testing in a short ragweed sensitive individual, for the presence of free antigen.

Results. Each of the 5 antigens was exposed to tannic acid treated sheep erythrocytes (S RBC) prepared with sterile precautions. To test the possibility that a polysaccharide antigen may be active, the antigens were also exposed to untanned sheep RBC (7). These various adsorbed antigen preparations were added to rabbit-anti-SR serum diluted serially. The results of the hemagglutination with the 5 antigens adsorbed onto untanned sheep RBC were uniformly negative and are not recorded. However, washes from the supernates of these tests were active in skin tests in SR sensitive individuals. Table I records the results obtained in the tannic acid type hemagglutination tests with antigens prepared as above, and stabilized with normal rabbit serum 1:200. There are 2 readings for each antigen in Table I. After reading the pattern at the end of 2 hours' incubation, the tubes were shaken and allowed to resettle for 36 hours. If antigen was eluting from the cells in sufficient quantity it should

inhibit the agglutination. The data show a 16-fold decrease in titer with Antigen #2, and a 32-fold decrease with Antigen #4. Antigen #5, the supernate from trypsin digestion and 10% TCA precipitation, failed to show any hemagglutinating activity, indicating either loss of adsorptive property, or lack of antibody in the rabbit anti-SR-serum to this fraction, since this fraction showed skin reactivity in one SR sensitive subject and not in another.

A question raised is whether this decrease in titer shown in the table is due to free, eluted antigen competing with fixed antigen for the antibody. If eluted antigen was responsible, it would be possible to wash away the eluted antigen, and if enough antigen remained on the cells, they should again show agglutination to the original titer. So, the various tanned RBC-antigen preparations were allowed to incubate for 36 hours in saline suspensions. These were then mixed with serial serum dilutions. (These dilutions were aliquots of the master dilutions prepared 40 hours previously.) The titrations at 2 hours yielded titers identical to those shown in Table I for 36 hours. However, when aliquots of these cell suspensions held for 36 hours were washed and resuspended in fresh saline, and used in hemagglutination tests as before, the titer noted at the end of 2 hours' incubation was identical with the 2-hour titer recorded in Table I. Upon shaking these latter titrations and allowing a 36-hour incubation period, the titer again showed a decrease. These data indicate that the elution of antigen from the tanned-antigen-treated RBC is not a function of the contact of adsorbed antigen with antibody, but can occur in the absence of antibody.

To further prove the presence of eluted antigens in the supernates after 36 hrs incubation, the supernates were recovered and injected into the skin of a short ragweed sensitive individual whose skin was reactive to all 5 antigenic components in soluble form. Injection of 0.05 ml of each supernate showed immediate wheal and erythema reactions in 15 minutes. The intensity of these reactions was recorded, but since the subject had a greater or lesser sensitivity to each of the antigenic components, quantitation was impossible. However, the reactions did indicate that antigen eluted from each of the RBC preparations, and could be detected in the supernatant fluids.

Since the maximum hemagglutination titer is again seen after washing the cells free of eluted antigen, it is obvious that tanned RBC adsorb more antigen than is necessary to show hemagglutination.

Summary. (1) Pollen antigens (short ragweed) elute slowly from the surface of tanned sheep erythrocytes. (2) This elution does not apparently affect the titer obtained over a 2 hr incubation period. (3) Some antigenic fractions fix more permanently than others. (4) The elution is not a function of contact of the RBC-antigen with its specific antibody. (5) This method may possibly be used to separate various antigenic components in the search for antigens with fewer specificities.

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