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Density-Gradient Sedimentation of Skin-Sensitizing Antibody and β_2 A-Globulin in Serum of Allergic Individuals.* (29018)

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Two classes of antibodies, whose sedimentation coefficients are 7S and 19S respectively, have been recognized in many species including the human. The skin-sensitizing antibody (SSA) of human atopic hypersensitivity has not yet been clearly identified with either of these classes of antibody. Sehon has reported that ragweed SSA sediments with the 19S globulins(1). Stanworth(2) and Augustin(3) have observed that SSA to horse dander and grass pollen, respectively, appeared to be associated with the 7S globulins. Heimlich *et al* found equal activity in serum fractions enriched with either 7S or 19S globulins (4). Rockey and Kunkel localized the SSA in a glucagon-sensitive patient in a sedimentation zone intermediate between 7S and 19S (5).

Recently attention has been focused on the possibility that human SSA may be a β_2 A-globulin(6,7). The sedimentation coefficient of β_2 A-globulin has not been firmly established, but present evidence suggests that it is in the 7S class of globulins but with a tendency to form polymers resulting in complexes of the order of 9S to 13S(8). In this study we have investigated the sedimentation properties of both skin-sensitizing antibody and

β_2 A-globulin in the serum of patients allergic to ragweed pollen by sucrose density-gradient ultracentrifugation.

Materials and methods. For reference purposes purified and concentrated 7S and 19S globulins were sedimented separately in sucrose density gradients. The 7S fraction used for this purpose was immunoelectrophoretically pure gamma globulin. It was prepared from normal serum by Na_2SO_4 precipitation followed by starch-block electrophoresis. The starch-block eluates were further purified by ion-exchange chromatography on a DEAE-cellulose column. The 19S fraction was obtained by gel filtration of serum on a Sephadex G-200 column. 0.2 ml samples of each purified fraction were layered over 5.0 ml sucrose gradients and centrifuged for 18 hours at 35,000 rpm in a Spinco model L ultracentrifuge using the SW-39 swinging bucket rotor. Thirty successive fractions were collected from a hole punctured in the bottom of the tube and assayed for protein spectrophotometrically at 280 $\text{m}\mu$.

0.2 ml samples of serum from 4 untreated ragweed-sensitive patients were sedimented under conditions identical to the reference samples. The resulting fractions were assayed for: 1) protein, 2) skin-sensitizing antibody by the Prausnitz-Küstner (P-K)

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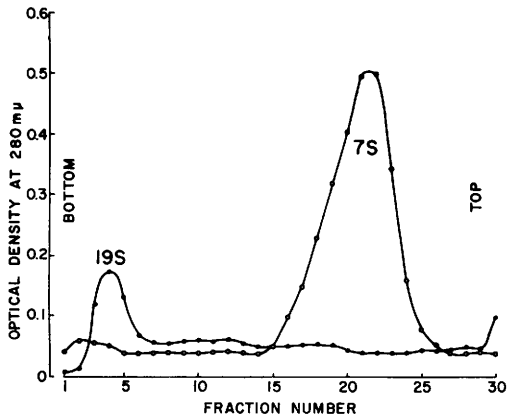


FIG. 1. Density-gradient sedimentation of purified 19S and 7S gamma globulin fractions.

passive transfer technique using dwarf ragweed extract 1:500 to challenge the skin sites, and 3) β_2 A-globulin, by reacting serial 2-fold dilutions of each fraction with specific goat anti-human β_2 A-antiserum in micro-Ouchterlony tests on standard microscope

slides. The highest dilution giving a detectable precipitin line was taken as the " β_2 A titer" for that fraction.

Results. Fig. 1 shows the density-gradient sedimentation of the purified 19S and 7S globulin fractions from normal serum. The density-gradient sedimentation of the 4 allergic sera is shown in Fig. 2. In the whole-serum samples the concentration of 19S globulin is too low to be detected as a peak on the protein curve, and in most cases the 7S globulins and 4S albumin tend to fuse into a single broad peak. The skin-sensitizing activity, indicated by the upper bar-graph in each diagram, shows sedimentation characteristics distinctly slower than the purified 19S globulin of the reference sample. Furthermore, the sedimentation of the bulk of the skin-sensitizing activity is faster than that of the purified 7S globulin fraction, although in 2 of the sera (C.W. and M.H.)

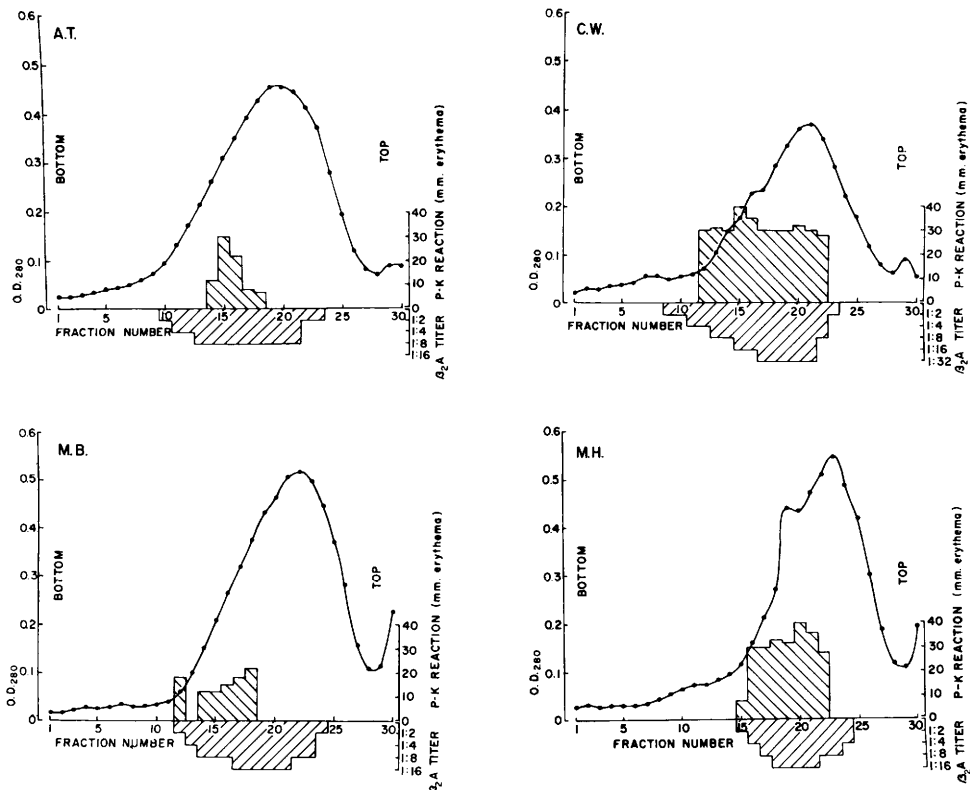


FIG. 2. Density-gradient sedimentation of 4 allergic sera showing distribution of total protein, skin-sensitizing activity (P-K reaction), and β_2 A-globulin.

some skin-sensitizing activity is present in the 7S region also.

β_2 A-globulin, indicated by the lower bar-graphs, is present in all fractions which contain skin-sensitizing antibody. However, in all 4 sera β_2 A-globulin extends into the lighter 7S region as well.

Discussion. These data indicate that SSA in the serum of patients allergic to ragweed has sedimentation properties intermediate between 7S and 19S globulins. Rockey and Kunkel have reported similar results in a rather unusual case of sensitivity to glucagon, presumably induced by glucagon-contaminated insulin injections in their patient (5). It is possible therefore that the intermediate molecular size may be a property associated with the ability of such antibodies to sensitize human skin, and perhaps other tissues as well.

Furthermore, the skin-sensitizing activity and β_2 A-globulin in these sera show similar sedimentation characteristics, which is in accord with the present concept that human SSA may be a β_2 A-globulin(6,7). The recent finding by Leddy *et al*(9) that *in vitro* treatment of allergic serum with sulfhydryl reducing agents destroys skin-sensitizing activity is in accord with this concept, since such treatment is known to dissociate 10S β_2 A-

globulin molecules into 7S sub-units(10).

Summary. The molecular size of the skin-sensitizing antibody in the serum of 4 ragweed-sensitive patients was studied, using the method of sucrose density-gradient sedimentation. The skin-sensitizing activity was present in a protein fraction of serum which sedimented somewhat faster than the 7S globulins but definitely slower than the 19S globulins. The skin-sensitizing antibody was associated with β_2 A-globulin, although the latter extended into the lighter 7S region.

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Urinary Steroids in Neurotic- and Manic-Depression.* (29019)

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Significant elevations in urinary 17-ketogenic steroids (17-KGS) with relatively low levels of urinary 17-hydroxycorticosteroids

(17-OHCS) have been reported in adreno-genital syndrome(1), Cushing's syndrome(2), and recently in patients with adrenocortical hyperplasia, carcinoma, and "idiopathic" hirsutism(3). In this paper similar findings for groups of neurotic- and manic-depressive patients are presented, and a significant correlation between the severity of depressive symptomatology in those patients and their excretion of 17-KGS is reported.

Materials and methods. Numerous 24-hour

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