feeding of the 20% dehydrated alfalfa meal in the absence of the antioxidant. The responses obtained with these 2 ethoxyquin treatments amounted to 89% of that obtained with the antioxidant in the diet.

The loss of dietary xanthophylls during the experimental period amounted to 12% in the absence of ethoxyquin. These data suggest that the oxidative destruction of xanthophylls in the digestive tract of the laying hen was negligible since neither oral nor parenteral administration of ethoxyquin resulted in identical levels of yolk pigmentation. However, the possibility exists that the injected antioxidant was excreted into the gut and thereby served to protect the xanthophylls.

Summary. Three experiments have been conducted to evaluate the site of action and effectiveness of the antioxidant ethoxyquin on egg yolk pigmentation. Results of the first experiment conducted during a period of high ambient temperatures indicated an average improvement of 53.8% in yolk pigmentation due to the feeding of 125 ppm of the antioxidant in addition to dehydrated alfalfa meal in the diet of laying hens. In the absence of ethoxyquin, dietary loss of xanthophylls amounted to 24% and loss of beta-carotene was 32%.

The second experiment conducted during cooler weather resulted in an improvement of 21% in yolk pigmentation due to ethoxyquin feeding. Dietary losses of carotenoids in the second study were negligible.

The result of the third experiment indicated approximately 10% of the xanthophylls were destroyed *in vivo*. This *in vivo* loss of xanthophyll pigments was prevented by either the feeding of 125 ppm ethoxyquin in the diet or administration of the antioxidant by capsule (50 mg, 3 times daily) or intramuscular injection (50 mg, 3 times daily).

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β_{2A} and Other Immunoglobulins in Isolated Anti-A Antibodies.* (28341)

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A considerable body of evidence indicates that the β_{2A} globulins probably represent a third class of antibody proteins in addition to the well-established 7S and 19S γ-globulins(1,2). However, the clear delineation of antibody activity in this serum protein fraction has proven very difficult primarily because of the problems involved in isolating β_{2A} globulins which are completely free of

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ordinary 7S γ-globulins. Early preparations of Schultze(3) showed the presence of a number of antibody activities, but these fractions were admittedly contaminated. More recently, Heremans and Vaerman(4) and Fireman *et al.*(5) have presented evidence for the occurrence of human skin-sensitizing antibodies in this fraction.

The finding of a number of isoagglutinins with sedimentation rates between those of 7S and 19S antibodies and with unusual chromatographic properties(6) raised the possibility that these antibodies might belong to the β_{2A} class. To explore this question, specific precipitates were prepared with A substance from high-titer anti-A sera, and their antigenic character determined. Although the bulk of the intermediately sedimenting antibodies did not appear to be of this class, varying amounts of β_{2A} proteins were found in specific precipitates from different individuals.

Material and methods. Sera. High-titer anti-A sera were obtained primarily from Knickerbocker Blood Bank and Ortho Laboratories from individuals who had received injections of hog gastric A substance. One serum (R.G.) was kindly furnished by Dr. Richard Rosenfield from a mother immunized in pregnancy. It was from a Type O individual. The remainder of the sera were from Type B individuals.

Specific precipitates were prepared from sera heated at 56°C for 30 min. In some instances the sera were also absorbed with aggregated γ-globulin primarily to remove the various anti-γ-globulin factors. A highly purified preparation of A substance, furnished by Dr. Walter Goebbel, was utilized throughout this work. Precipitin curve analysis of 8 sera showed from 30-150 μg antibody nitrogen per ml. Equivalence point precipitates were obtained after 24 hours incubation at 4°C. These were washed 4 times with isotonic saline and immediately dissolved either in excess A substance or in sodium acetate buffer, pH 3.8. In no instance did the precipitate dissolve completely, but in the sera studied most extensively, up to 70% dissolved in the acid buffer. The latter eluate following treatment with periodate, showed approximately the expected recovery of activity. A substance was added to the precipitates in amounts more than 10 times equivalence.

Rabbit antisera. Three highly specific β_{2A} antisera were employed. Two (380 and 462) were prepared by immunizing rabbits with highly purified preparations of β_{2A} myeloma proteins(2). The third (525) was prepared against a γ_1 fraction from normal serum, purified by Zn precipitation and zone electrophoresis. All showed heavy lines by immunoelectrophoresis with normal serum for the β_{2A} component.

Antiserum 380 was unusual in that it showed almost no reaction with 7S γ-globulin and could be used in the unabsorbed state for detection of β_{2A}-globulin. Occasionally, in Ouchterlony plate analysis, a weak 7S γ-globulin line appeared after prolonged incubation. Antiserum 462 was made specific for β_{2A} by absorption with Fr 11 γ-globulin. It gave a pronounced double line with 7S γ-globulin prior to absorption. Antiserum 525 contained multiple lines and was made specific for β_{2A} only by absorption with serum R.J., which was shown previously to be completely devoid of β_{2A} proteins(7). The 3 antisera, following these absorption procedures, identified the same single line in a variety of normal sera and served to identify β_{2A} myeloma proteins.

The other antisera were prepared as previously described from this laboratory. Agar plate analysis and immunoelectrophoresis were carried out as previously described(2).

Results. The specific precipitates from 8 different sera, following solubilization with excess A substance, were studied by Ouchterlony plate analysis with antisera against 7S γ-globulin, 19S γ-globulin, and β_{2A}-globulin. One such experiment is illustrated in Fig. 1 with an A excess eluate. It indicates primarily that this specific precipitate contained definite β_{2A}-globulin and gave strong confluent lines with 2 highly specific antisera. This and other experiments also showed the presence of 7S γ-globulin and 19S γ-globulin. The latter is visible as a faint curved line near the antigen well. The

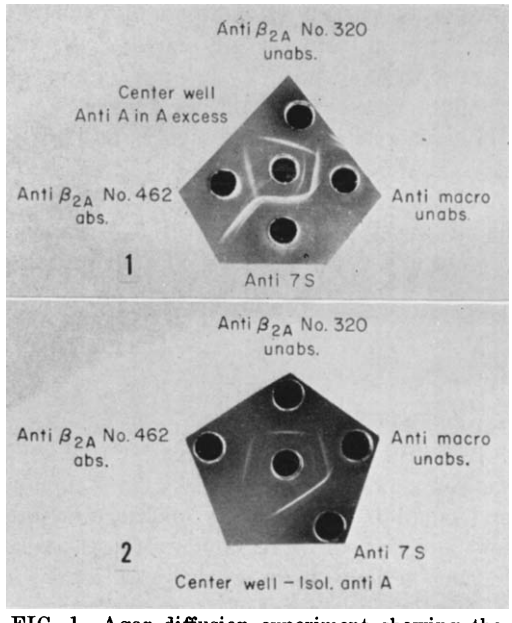

FIG. 1. Agar diffusion experiment showing the reaction of A-anti-A specific precipitates solubilized in excess A substance with various antisera. Distinct β_{2A} lines are apparent with the β_{2A} antisera.

FIG. 2. The reaction of isolated anti-A antibody with various antisera showing the presence of all 3 classes of immunoglobulins.

specific precipitates from 6 of the 8 sera showed good β_{2A} lines similar to those illustrated in Fig. 1, with the 3 β_{2A} antisera described under Methods. All contained considerable 7S γ-globulin and all but one contained 19S γ-globulin. The one serum lacking a macroglobulin line also failed to show activity in this component on density gradient ultracentrifugal analysis.

The β_{2A} lines were also visible with antibody solutions prepared by acid elution of specific precipitates and were given by all 3 specific antisera. Fig. 2 illustrates a second agar plate experiment with the acid eluate of the specific precipitate from a different serum than that shown in Fig. 1. Quantitative precipitin analysis with absorbed antisera for determination of the relative amount of β_{2A}-globulin in acid eluates indicated that the majority of antibodies in most of these sera belonged antigenically to the ordinary 7S γ-globulin type. The β_{2A} content, determined in 2 acid eluates, ranged from 8-15% of the total protein.

Immunoelectrophoresis experiments with both types of eluates showed a β_{2A} line in its characteristic position. These experiments proved more satisfactory with the acid eluates because large amounts of protein remained around the well with the A excess eluates. However, since β_{2A} proteins were retarded in mobility by the A substance, further evidence was furnished for a specific combination with antigen.

Antisera were made against the acid eluates of all 8 of these sera as part of another study. A large number of these antisera showed antibodies to β_{2A}-globulins and several, after absorption with R.J. serum, proved useful specific reagents for routine laboratory analysis for β_{2A}-globulin.

The possibility that the β_{2A} might represent some type of anti-γ-globulin factor or complement-like component which bound to antigen-antibody precipitates was considered. However, thorough prior absorption of one of the sera in the unheated state with aggregated γ-globulin under conditions which removed complement activity, failed to decrease the β_{2A}-globulin found in the specific precipitate with A substance. Heating the sera at 56°C had no effect on the appearance of β_{2A}-globulin in the precipitate.

Discussion. The present studies clearly indicate the presence of β_{2A}-globulin in the specific A-anti-A precipitates from most high-titer sera. The possibility of a complement component or some anti-γ-globulin factor coprecipitating with the antigen-antibody complex, appears remote as an explanation of these observations because absorption of serum with aggregated γ-globulin failed to remove the β_{2A} lines under conditions known to remove such factors(8). The accumulated evidence indicates that the β_{2A}-globulin found represented antibody. Unfortunately, no serum has been found where the specific precipitate consists exclusively of β_{2A}-globulin. It is worth searching further for such a serum in order to gain information on special characteristics of such precipitin reactions.

The use of antigen excess dissolved precipitates for analysis of antigenic components of antibody precipitates proved very valuable

in agar diffusion experiments and even in immunoelectrophoresis. All 3 classes of immunoglobulins were readily detected. In all instances 7S γ-globulin proved to be the dominant antigen, but 19S γ-globulin and β_{2A}-globulin were readily demonstrated. These results correspond closely to the studies of McDuffie *et al.*(9) on anti-A antibodies. They described 3 types of antibodies, following immunization with isolated anti-A, which relate to the 3 immunoglobulins found in the present study. Their undetermined component probably was the β_{2A}-globulin.

The intermediate sedimenting isoagglutinins previously described(6) proved to be largely polymers of what from the antigenic standpoint appeared to be ordinary 7S γ-globulin. These findings will be described elsewhere(10). The question of the exact s-rate of the reactive β_{2A}-globulins was not readily determined. In the acid eluates the major portion sedimented with an s-rate of approximately 7S but the possibility exists that this treatment may have caused some dissociation.

Summary. 1. The specific precipitates prepared with A substance from 6 out of 8 high-titer anti-A antisera showed the presence of β_{2A}-globulin. This was demonstrated with 3 different antisera specific for this component, utilizing eluates prepared by excess A substance and also by acid extraction. 2. Antisera made against eluates of specific precipitates contained antibodies specific for normal β_{2A}-globulins. 3. All 3 classes of immunoglobulins were encountered in the specific precipitates of the majority of the sera. Antibodies corresponding in antigenic type to ordinary 7S γ-globulins, made up the bulk of the precipitates studied.

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Concentration of Adenoviruses by Isoelectric Precipitation. (28342)

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Various methods for virus concentration were examined during efforts to increase antigenic potency of adenovirus vaccines. Such conventional methods as centrifugation, dialysis, and precipitation in salt solutions of varying saturation resulted in unsatisfactory virus concentration or in inclusion of large amounts of non-viral protein. However, it was observed that virus could be precipitated selectively by proper adjustment of pH with high virus recovery on resuspension. Steinman and Murtaugh(1) already have reported that a simian adenovirus and its complement-fixing antigen could be concentrated from culture fluids by this technique. These observations have now been extended to adenovirus types of human origin which are candidates for inclusion in vaccines.

Methods. Adenovirus strains used for most experiments were Types 1 (Ind. 1), 2 (Ind. 2), 3 (J.F.), 5 (Ad. 75), and 7 (L.L.) from Dr. R. J. Huebner, Nat. Inst. of Health, Bethesda, Md., and Type 4 (R-Wallace line Q) from Lt. Col. E. Buescher, Walter Reed

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