TC) was also demonstrated when administered to birds experiencing an acute outbreak of another respiratory disease. In Exp. 4, 2500 12-week-old pullets were each injected with 0.20 ml of the vaccine and compared with an equal number of nonvaccinated birds under the same managemental conditions. Over a period of 12 days, no difference was observed in symptomatology, weight gains, or feed consumption between the two groups. Failure of symptoms to become aggravated was also observed in Exp. 2, in which the vaccination was conducted during an acute respiratory phase of infectious bronchitis induced by vaccination for the disease 10 days previously.

Summary. 1) Newcastle disease virus was modified by serial passage of the California 11914 strain through tissue cultures using minced chicken embryos suspended in Simm-Sanders medium. 2) The modified virus was administered intramuscularly to over 20,000 chickens of all ages without producing any nervous or respiratory symptoms, but produced an immunity as demonstrated serologically and by intramuscular challenge with a virulent strain of ND virus. One dose of the vaccine given to susceptible chicks at 5 days of age or over induced an immunity lasting at least 12 weeks. Two doses from 7 to 9 weeks apart have resulted in a high degree of immunity which was still evident 33 weeks later. 3) When administered to 2 flocks of chickens, one in acute stage of infectious bronchitis and the other with chronic respiratory disease, the virus apparently did not increase the severity of the respiratory disease condition. 4) Repeated trials showed that vaccinated chickens did not transmit the infection to unvaccinated susceptible birds within the same pen during 33 weeks. 5) The modified virus can be propagated on HeLa cells to produce a vaccine for poultry, thus avoiding the introduction of egg-borne infections to poultry flocks through vaccines produced in chicken embryonated eggs.

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Some Physical and Chemical Properties of Antibody Against Complement.* (23409)

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We(1) have previously described an antibody against complement. Most of the experiments then reported were concerned with activity of anticomplement as an inhibitor in the immune hemolytic system. Mention was made, however, of experiments to determine more of the physical and chemical properties of this antibody. The purpose of this paper is to describe in more detail studies on quantitative antibody protein, antibody expressed as carbon-14, Tiselius electrophoretic analyses, and fractionation of anticomplement-containing sera.

Methods. Antibody was prepared in rabbits to complement of the guinea pig, human being, monkey, and mouse by the method previously described(1). Briefly, the immunizing procedure consisted of giving animals several subcutaneous injections of different immune precipitates to which complement had

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TABLE I. Anticomplement Protein Determination.

Material analyzed	Avg total protein (mg)	Difference from ppt.	Diff. from ppt. + C′
Precipitate* alone	5.60		
" + C′†	5.90	+.30	
" + △C′‡	5.70	+.10	−.20
" + C′ + RAGPC′§	6.25	+.65	+.35
" + △C′ + RAGPC′	5.85	+.25	−.05

* Bovine serum albumin + anti-bovine albumin. † Complement. ‡ Complement heated at 56°C, 1 hr. § Rabbit anti-guinea pig complement.

been affixed, incorporated in an adjuvant.

Since antibody to complement was prepared by immunization of animals with immune precipitates to which complement was adsorbed, this antibody should combine specifically with such complexes and be measurable as increased protein in the aggregate. On the other hand, if precipitates were treated with heat inactivated complement, no specific complement protein, but only nonspecific protein combination, should be measurable, as shown by Heidelberger(2). Combination of anticomplement with the latter complexes should therefore reflect nonspecific protein binding from antibody-containing sera. The difference should give a quantitative measure of anticomplement uptake in a given set of experimental conditions.

For the expression of anticomplement as antibody protein, a modification of the method used by Heidelberger(2) for determination of complement as combining nitrogen was used. Precipitates were prepared from crystalline bovine serum albumin and rabbit anti-bovine albumin in the zone of slight antibody excess. Triplicate sets of tubes were used for each variable. After allowing the precipitates to form for 15 minutes at room temperature, neutralized fresh guinea pig complement, complement heated at 56°C for 60 minutes, or 0.15 M NaCl was added to each set of tubes. The mixtures were then incubated for 16 hours at 4°C. Anticomplement or saline was then added. Following incubation for 30 minutes at room temperature, all tubes were centrifuged 30 minutes at 4°C and 3,000 rpm. Precipitates were washed 3 times with cold 0.15 M NaCl under the same conditions of centrifugation. After the final washing, sediment in each tube was resuspended in saline, and quantitative biuret determinations made by the method of Gornall, Bordawill, and David(3).

Typical results of experiments with anti-guinea pig complement are described in Table I. The increase in protein due to combination of complement with the precipitate is 0.3 mg/3.0 ml guinea pig serum. Of this amount, 0.1 mg would be due to nonspecific adsorption, as evidenced by the combination of heated complement. Addition of 2 ml of anticomplement to the precipitate-complement complex accounted for the addition of 0.25 mg more protein. If heated complement were used, however, the amount of non-specific protein combination from anticomplement-containing sera amounted to 0.15 mg protein.

Correcting for non-specific adsorption and for volume of materials added, one finds that the total amount of complement protein bound to the precipitate is 0.067 mg/ml of guinea pig serum. The amount of antibody protein fixed to the precipitate-complement complex, on the other hand, is 0.1 mg/ml of antiserum.

Another means of showing the combination of anticomplement with its specific antigen would be measurement of uptake of radioactivity if the antibody could be appropriately labeled. Preliminary experiments(1) showed that carbon-14 labeled antibody which had combined with the complement-precipitate complex gave counts significantly above those obtained when C^{14} labeled normal rabbit serum was used for control. Non-specific adsorption of C^{14} from other serum fractions caused counts to be considerably above background in control tests, however.

In the present experiments, rabbits immunized against guinea pig complement were maintained for approximately 3 months to al-

TABLE II. Anticomplement Expressed as Carbon-14.

Property measured	Ppt + C'* + RAGPC'(C^{14})†	Ppt + C' + NRS(C^{14})‡	Difference
Total carbon, mg	2.793 (2.780)§ 2.784 2.752	2.584 (2.584) 2.618 2.550	.196
Counts/min. (corr.)	203.4 (204.9) 210.2 201.0	109.1 (118.7) 129.7 117.2	86.2 (72%)
Counts/min./mg C	72.8 (73.8) 75.5 73.0	42.2 (45.9) 49.5 45.9	27.9 (60%)

* Precipitate + complement. † Rabbit anti-guinea pig complement. ‡ Normal rabbit serum. § Values in parentheses are avg.

low a decline of antibody titer. Following this period each animal was given a "booster" injection of homologous complement antigen subcutaneously without adjuvant. Five days later, when the anamnestic response was in the ascending phase, each immunized animal and 2 normal control rabbits were given 9.18 mg of glycine-l-C^{14} by intravenous injection. The glycine contained a specific activity of 13.8 μc/mg. Six hours after receipt of the radioactive glycine all animals were exsanguinated by cardiac puncture and their serum separated for study. Both the serum containing labeled anti-guinea pig complement and the labeled normal rabbit serum were fractionated in a Misco Electrochromatography Apparatus to minimize non-specific adsorption. Material from tubes containing high anticomplement activity as measured by inhibition of immune hemolysis was pooled, as was material from the same tubes containing fractions of normal serum. Precipitates were prepared in the same manner as previously described for protein determination, and 1.0 ml of guinea pig complement was adsorbed to the precipitate in each tube. Aliquot portions of either the pooled fractions of anticomplement or normal rabbit serum were added. The precipitates were allowed to incubate overnight and then washed and centrifuged. Each precipitate was analyzed for total carbon and carbon-14 activity by the gas phase counting method of Van Slyke, Steele, and Plazin(4).

The results of studies on C^{14} uptake by precipitate-complement complexes from fractions of such sera are presented in Table II. The average increase in total carbon uptake from anticomplement-containing sera is 0.196 mg above the uptake from normal serum. The average increase in carbon counts per minute is 86.2, or a 72% increase.

Even after separation the amount of C^{14} uptake from normal rabbit serum (172.8 counts/min, uncorrected) was considerably above the average background (72.9 counts/min.). The count for precipitate-complement complexes to which anticomplement was affixed (250.5 counts/min, uncorrected), was sufficiently increased to rule out the possibility of non-specific adsorption as the cause of the elevation in C^{14} activity.

Additional evidence in support of the characterization of anticomplement as true antibody would be the changes in serum protein content following immunization with complement antigens. Serological studies to detect antibodies for soluble protein antigens used to prepare the precipitates and for guinea pig serum were negative. The only observed change in sera of such animals was a rise in complement-inhibiting ability.

Tiselius electrophoretic studies were performed on sera of individual rabbits immunized against guinea pig, mouse, and human complement, before and after the immunizing process. A Perkin-Elmer Electrophoretic Apparatus was used. Barbital buffer, pH 8.6, ionic strength 0.1, resistance 330 ohms, was employed for all determinations. The time of analysis was 70 minutes, the current 10 ma, and the temperature 1°C.

The percentage values for each protein fraction before and after immunization in individual animals are given in Table III. An increase between 8.7 and 10.8% in γ

TABLE III. Electrophoretic Percentages of Protein Fractions before and after Immunization.

Fraction	RAGPC'* Pre	Post	% change	RAHC'† Pre	Post	% change	RAMC'‡ Pre	Post	% change
Albumin	51.3	38.4	−12.9	57.6	57.1	− .5	57.7	48.0	− 9.7
α_1 globulin	7.1	6.6	− .5	4.9	2.7	−2.2	5.0	4.5	− .5
α_2 "	18.6	14.8	− 3.8	6.7	6.5	− .2	6.7	7.8	+ 1.1
β "	16.8	24.0	+ 7.2	19.9	14.1	−5.8	21.8	20.1	− 1.7
γ "	6.2	16.2	+10	10.9	19.6	+8.7	8.8	19.6	+10.8

* Rabbit anti-guinea pig complement. † Rabbit anti-human complement. ‡ Rabbit anti-mouse complement.

globulin occurred in the serum of each animal following immunization. In the rabbit anti-guinea pig complement, an increase in β globulin was also noted which appears to be due to the concentration of hemoglobin in this area. Although we realize that numerous factors may affect the γ globulin concentration, the consistent increase of this fraction in these animals following immunization in the absence of other obvious stimuli would tend to support the nature of anticomplement as true antibody.

For certain analytical procedures (carbon-14-analysis), and for *in vivo* studies, it was necessary to obtain anticomplement in a purified and concentrated form. Fractionation of anticomplement-containing sera was carried out in a Karler-Misco Electrochromatography Apparatus using 0.01 M barbital buffer, pH 8.6, ionic strength 0.01, at room temperature. The rate of serum input was 0.5 ml per hour. The total fluid volume in each fraction tube was estimated. Total protein per fraction was determined by the quantitative biuret method(3). The antibody titer of each fraction was measured by its ability to prevent 2 full units of complement from hemolysing sensitized sheep erythrocytes.

The efficiency of protein recovery in the process was 54.4% of total input. Antibody was demonstrable to some extent in 13 of the 22 fractions recovered. These contained 64.8% of the recovered protein. However, most of the antibody activity was found in 9 fractions containing 45.6% of the protein. As expected, maximum activity occurred in the fractions where γ globulin would normally separate out.

Summary. 1) Our data indicate that antibody against complement can be expressed as protein in specific combination with an immune precipitate to which complement has been affixed. 2) Anti-complement which has been labeled by injection of glycine-l-C^{14} into animals during active antibody production can be demonstrated as carbon-14 bound to precipitate-complement complexes. 3) Electrophoretic analysis of the sera of rabbits before and after immunization with guinea pig, human, and mouse complement shows that a consistent rise in γ globulin occurs during the immunization process. 4) Finally, electrochromatographic separation of antibody containing sera proved to be an effective means of concentration and partial purification of the antibody.

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