trifugation revealed that the rapidly sedimenting peak of antibody corresponded to the 2-ME resistant antibody. The nature of the 2-ME sensitive antibody and the significance of its detection in diagnosis of the viral infection were discussed.

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Immunophoretic Evaluation of Blood Serum Proteins in Chickens. I. Changing Protein Patterns in Chickens According to Age.* (31238)

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The identification of some protein fractions in immunophoretic preparations of chicken blood serum can be readily made by analogy from similar preparations of human blood serum. Other fractions may be tentatively identified pending verification by ultracentrifugation methods. We have used this principle in analyzing serum protein profiles in normal and experimental chicks subjected to vit E-deficient diets which produce selectively nutritional encephalomalacia or myopathy, and in chickens with genetic muscular dystrophy. Since variations in the pattern were observed in chickens of varying ages, a systematic examination of patterns was made in a series of normal chickens, and the data are presented here.

Methods and materials. Three sets of chickens were studied in 2 separate laboratories: (1) 25 chickens, received at one day of age, of the Arbor Acre strain. (2) 4 chickens of the New Hampshire strain, also used as controls for the chickens with genetic muscular dystrophy. (3) 13 chickens obtained from Cuxhaven, Germany consisting mostly of White Leghorn chicks. These chicks were fed for the first 3 weeks on a commercial preparation for newly hatched chicks.† Afterwards, the New Hampshire chicks were fed on a balanced commercial feed‡ while the Cuxhaven chickens were fed a stock feed suitable for adequate growth and development, and containing a mixture of fish and cereal meal. All chickens were kept in cages which did not permit free mobility to the older chickens. The sex of the older chickens was recorded, and of the younger ones, only those of the Arbor Acre strain were known.

Blood samples were obtained either by ventricular puncture under sodium pentathol anesthesia (100 mg/kg I.P.) from cervical

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† Starteena—Ralston Purina Co., St. Louis, Mo. For the Cuxhaven chicks, a preparation called "Volikrafthorn K Kuken Alleinfutter," by Bergesche Kraftfutterwerke, H. Schmidt, K. G., 4 Dusseldorf 1.

‡ Groweena—Ralston Purina Co., St. Louis, Mo.

TABLE I. Serum Protein Profile in Normal Chicken Serum According to Age.

Age, days	No.	Prealbumin	Albumin	Fast α globulin	α_1 lipoprotein	β lipoprotein	Macro$_2$ globulin	β globulin	Transferrin	1A γ globulin	1M γ globulin	7S γ globulin	Other γ globulin
1	2	++	++	++	++	+	++	±	0	++	++	++	++
4	3	++	++	+	+	+	+	±	+	++	++	++	
6	3	++	++	+	+	+	±	+	+	++	++	++	
8	3	++	++	+	+	+	+	+	±	++	++	++	
10	1	+	++	++	+	0	+	±	±	++	++	++	
11	1	+	++	+	+	0	+	±	±	++	++	++	
12	3	+	++	++	+	0	+	++	+	++	++	++	±
13	2	0	++	++	+	0	+	++	+	++	++	++	±
15	2	+	++	+	+	0	+	+	+	++	++	++	
18	1	±	++	++	+	0	+	±	0	++	++	++	
20	2	±	++	++	+	0	+	±	±	++	++	++	±
21	1	0	++	+	+	0	+	±	+	++	++	++	
56	3	±	++	+	+	0	+	+	±	++	++	++	±
70	1	0	++	+	+	0	+	±	0	++	++	++	
84	3	0	++	+	+	0	+	++	+	++	++	++	+
220	3	0	++	++	+++	0	++	++	++	++	++	++	+

vessels after decapitation or from a wing vein without anesthesia from older chickens. The use of the anesthesia did not appear to affect the findings. Blood was generally obtained after a 24-hour fast, except from the Cuxhaven group. The variation in this procedure also appears not to have influenced the protein profile of the serum.

Serum was separated from the clot by slow centrifugation. Fractions were separated by electrophoresis on agar gel according to the method of Wieme(1), in barbital buffer at pH 8.6, ionic strength = 0.1, in an L KB 6800A electrophoretic cell for one hour at 250 V and 50 M. A. One micromilliliter of serum was used. Subsequently immunophoresis was employed according to the modification by Scheidegger(2) of Grabar and Williams'(3) method. Antibodies used consisted largely of that prepared by immunizing rabbits against normal mature chicken serum following the method of Grabar(4). Occasionally a commercially prepared§ antiserum was used, which gave identical results with that produced by us.

Immunophoretic preparations were made two to a slide, each providing a check for the other. Frequently serum from a normal chicken was paired with that of an experimental chicken. In this paper the findings in a series of healthy chickens from one day old to 210 days of age will be summarized and discussed. Those from experimental chickens will be presented in comparison reports.

Results. A summary of the serum protein profile is tabulated in Table I. When data from more than one chick were summarized, the results were averaged. ++ represented usual levels, + decrease, ± trace, and 0 absence.

In Fig. 1, a photograph of an immunophoretic preparation at 5 days is presented, and the curves are labelled in accordance with similar preparations from human serum. Since certainty about the identity of some curves was impossible, they are labelled generally, *e.g.*, fast alpha globulins, beta globulins, etc. Beta lipoprotein curve cannot be separated from alpha$_2$ lipoprotein, hence they are grouped together. Whether this particular curve really represents the lighter lipoprotein or not is currently being investigated by ultracentrifugation flotation methods.

The most constant alterations with age occur in the prealbumin and with the precipitate in the position of the beta lipoprotein fractions. Both of these protein elements are present abundantly in preparations of the 1-8-day-old group. Beginning with the tenth day, prealbumin precipitation curve becomes less prominent, and after the 20th day is no longer present. Similarly the apparent beta

§ Obtained from Hyland Co., Los Angeles, Calif.

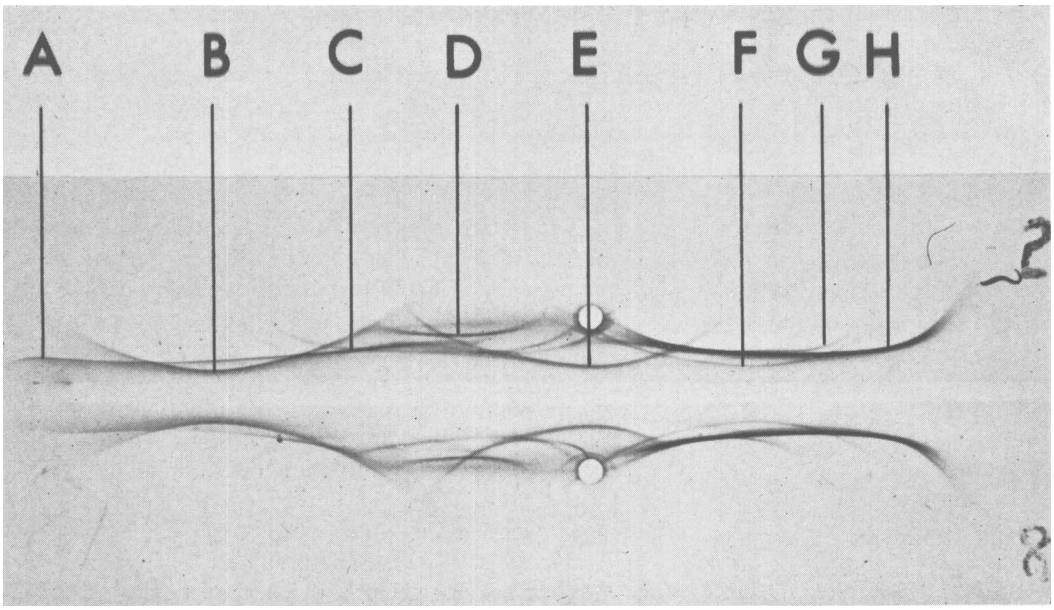

FIG. 1. Immunophoretic tracings from blood serum of two 5-day-old normal chicks. Antigen-antibody precipitation curves are designated according to those used for comparable curves in human blood serum. A. Prealbumin, B. Albumin, C. Alpha$_1$ lipoprotein, D. Beta lipoprotein, E. Transferrin, F. IA Gamma globulin, G. IM Gamma globulin, H. 7S Gamma globulin.

lipoprotein precipitate diappears after eighth day in normal chickens.

Gamma globulin was observed to appear as 4 fractions in the two one-day-old chicks. Thereafter, the most characteristic pattern consisted of 3 fractions, corresponding to 1A, 1M, and 7S gamma globulin. In older chickens a fourth fraction frequently appears, but this is not a constant finding.

Transferrin is a pronounced precipitate which exhibits little mobility away from the antigen well in younger chickens. In the older chickens, this curve is frequently absent. Other beta globulin fractions appear more prominently in older chickens.

Discussion. Asofsky *et al*(5) have studied the development of serum protein in chick embryos and in chicks up to 12 days of age by injecting labelled amino acids intraperitoneally and making autoradiographs of the immunophoretic preparations. Their findings that prealbumin is always present in embryonic and neonatal serum are confirmed by our data; further their autoradiographs demonstrate that this fraction is derived from egg yolk and is not synthesized except in ovulating hens. Our data fail to show its presence in ovulating hens, which may be due to the buffer and pH 8.6 which we used, since Marshal and Deutsch(6) failed to find this fraction using the veronal buffer at pH 8.6. Other investigators(7) do find this fraction in ovulating hens when borate buffer is used; however, some authors(8) claim that this fraction is not identical with that found in embryonic fluids.

Similarly, the gamma globulin fractions found in embryonic and neonatal sera are considered to be absorbed from the egg yolk, and it seems that the earliest synthesized gamma globulins fail to provide immune mechanisms. The increased number of gamma globulin fractions observed in the neonatal chickens in our experiments may well represent those provided by the egg yolk, whereas those appearing at later periods would probably represent synthesis related to the development of immune mechanisms.

Apparently, albumin, alpha and beta globulin fractions are synthesized embryonically and synthesis continues increasingly during the lifetime of the chicken. The albumin curve is readily recognized in our preparations, as well as are the fast moving alpha

and much slower moving beta fractions. In the former group can be seen the well-defined alpha$_1$ lipoprotein precipitation curve which becomes heavier and of slower mobility in ovulating hens, and in the latter group the apparent beta lipoprotein and the macro$_2$ alpha globulin positions can be readily identified, as well as the transferrin curve.

It must be emphasized that immunophoretic preparations depend upon optimal conditions for the precipitation of electrophoretically separated protein fractions by the diffusion of the appropriate antibody through the agar gel. Separation of the fractions depends on the medium used, the pH and the ionicity of the buffer, the voltage and amperage used in electrophoresis. The absence of a curve in the immunophoretic trace does not necessarily represent the absence of the fraction. However, the relative intensity of the precipitation curves in a series of preparations made under identical conditions is an indication of relative changes occurring in the various preparations. It becomes significant in our current studies, that no precipitation occurs in the position of the beta lipoprotein fraction as the chick ages. At least this suggests that this fraction decreases to a point that antibody antigen reaction does not take place. These observations are important in view of the findings to be reported in the immunophoretic patterns found in sera of chickens on vit E-deficient diets.

Summary and conclusions. 1. Immunophoresis was performed in a series of 42 normal chickens from ages 1 to 210 days. Rabbit serum immunized against normal mature chicken serum was used for antibodies. Barbital buffer at pH 8.6, ionic strength 0.1 was used. 2. Identification of fractions was attempted by designating precipitation curves in the chick serum preparation with the names used for curves of known fractions in identical position and of identical shape in human serum preparations made under identical conditions. 3. Significant findings in chicken serum indicate that all fractions are more abundant in neonatal life than at later periods. 4. Prealbumin becomes less abundant after the 12th day of life, and is not detected in the preparations after the 20th day. 5. Precipitation curve in the position of the beta lipoprotein fraction is not observed in preparations after the 8th day of life. 6. Additional fractions of gamma globulin appear in some preparations after the 12th day of life. 7. The findings are significant for comparison with serological protein profiles of chickens on vit E-deficient diets.

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