that the plaques of polio virus are completely inhibited or markedly reduced in size by specific antibody in the agar overlay. On the other hand, Black and Melnick(4) found that while the spread of poliovirus was inhibited by antibody under liquid overlay, the spread of herpes B virus was not inhibited. Such viruses as those of the herpesvirus group, as well as the poxviruses, may be ones for which this technic would be useful.

Summary. Incorporation of specific immune serum in the agar overlay does not inhibit the appearance or normal development of myxoma or fibroma virus plaques. On the contrary, these plaques are clearly visible on an unstained cell sheet; thus enabling an accurate and sensitive plaque count to be made with greater ease at least 2 or 3 days earlier than assays using the standard overlay. The accentuation of the plaques appeared to be due to 2 factors: (1) a precipitate in the agar around the plaque resulting from antibody-antigen reaction, and (2) an increased thickening of the cell layer at focus of infection. The former was directly proportional to the concentration of antibody in the overlay while the latter was related to the percentage of rabbit serum, either normal or immune, in the overlay. A clear distinction between plaques resulting from inoculation of an artificial mixture of myxoma and fibroma viruses was easily provided when fibroma antiserum was present in the overlay since the plaques differed both in size and density. It is suggested that technics reported here might be applicable to other viral assay systems.

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Serum Protein Synthesis by Embryonic and Neonatal Chicks. (27834)

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The chick embryo is an ideal organism for the study of serum protein synthesis since it is separated from the maternal circulation during its development. Heim and Schechtman(1) and others(2,3) have indicated that both qualitative and quantitative changes occur in the sera of developing embryos and neonatal chicks. It has not been determined what part, if any, of these changes is the result of protein synthesis by the animal, and what part is the result of absorption of proteins from the yolk(4). This problem has been investigated in our laboratory, using incorporation of radioactively labeled amino acid as a criterion for protein production, and autoradiography of immunoelectrophoretic patterns(5) to identify the labeled proteins.

Materials and methods. Animals. Seventeen- to nineteen-day-old chick embryos were given 100 μc ($\sim$50 μg) S-35 methionine intravenously; 3-day-old and 12-day-old chicks received 200 μc intraperitoneally. All animals were bled one day following injection.

Immunoelectrophoresis and autoradiography. Microimmunoelectrophoretic patterns(6) of experimental sera alone and mixed with chicken globulin (obtained by salt fractionation) or with adult chicken serum were prepared. Rabbit antisera against chicken globulin or against whole chicken serum were employed to develop the precipitation lines. The mixtures of experimental sera with "carrier" protein solutions were used in order to obtain reproducible immunoelectrophoretic patterns. It will be seen that the patterns obtained with experimental sera varied with the age of the animals. Autoradiographs were made as previously described(5).

Results. The antisera employed demon-

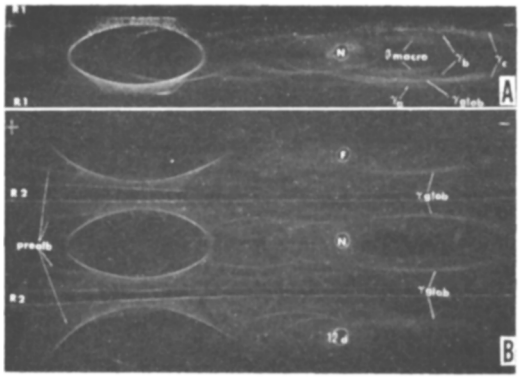

FIG. 1. Antigen wells: F—18-day embryo serum, added 2 ×; N—newborn chick serum; 12d—12-day-old chick serum. Antibody troughs: R1, R2—rabbit antisera against whole chicken serum. A. Typical immunoelectrophoretic pattern of newborn chick serum, showing the 3 unidentified proteins of γ mobility, γ_a, γ_b, γ_c. B. Note lower content of γ globulin at 12 days than at hatching. Prealbumin decreases progressively after hatching.

strated 5 proteins of gamma mobility (Fig. 1), in addition to a prealbumin, albumin, and several proteins in the α and β regions. One of these was the major γ-globulin, which has been shown to contain antibody activity(7). A second was the β_2-macroglobulin, verified by ultracentrifugation(8). The properties and function of the remaining 3 proteins are not known. The protein we have designated γ_b appears in these immunoelectrophoretic patterns as a line between the β_{2m} and γ lines, and parallel to the former. It is not present in the high molecular weight ultracentrifugal fraction of chicken serum.

γ-globulin and β_2-macroglobulin were both present in the sera of all animals. However, the concentration of β_{2m} was low, and could be detected only in the high molecular weight ultracentrifugal fraction. It was also noted that concentration of γ-globulin decreased from hatching until day 12. Prealbumin, which was prominent in embryo sera, was less conspicuous in neonatal sera.

The autoradiographs (Fig. 2) showed a labeling of albumin and of a few α and β proteins in the sera of all the experimental animals, with labeling of more proteins in the sera of neonatal than of the fetal chicks. Prealbumin was labeled in one serum, that of a 12-day-old chick. There was no labeling of either γ-globulin or of β_{2m}-globulin in any of the sera. However, the 3 other precipitation arcs in the γ region, namely γ_a, γ_b, and γ_c were labeled by most animals. γ_a was strongly labeled in all experimental sera. Very weakly labeled γ_b and γ_c were found in a few, but not all, embryo sera, while all neonatal sera showed moderate labeling of those lines.

Discussion. These experiments indicate that chick embryos and neonatal chicks produce, in addition to albumin and several α

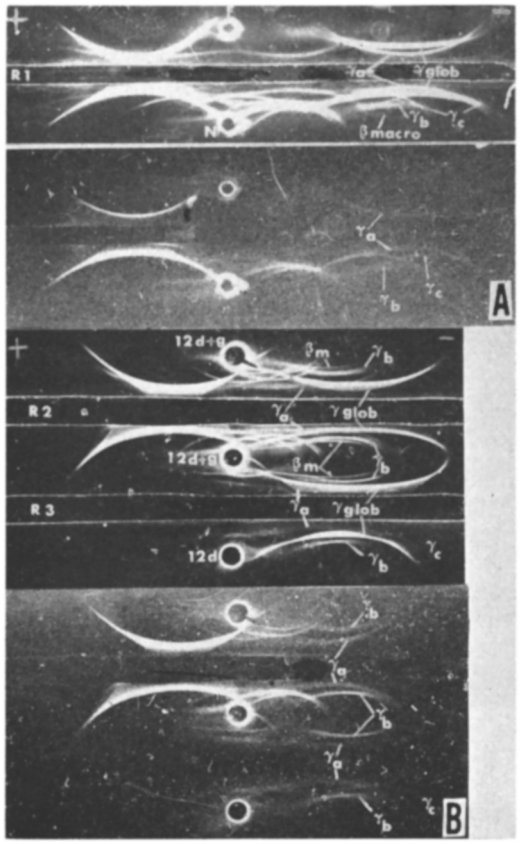

FIG. 2. Antigen wells: All sera are from animals given S35 methionine, added 2 or 3 × to the antigen well. F—18-day embryo; N—newborn chick; 12d—12-day-old chick; 12d + g—12-day-old chick with added normal gamma globulin. Antibody troughs: R1, R2 — rabbit antisera against whole chicken serum; R3 — rabbit antiserum against chicken globulin. A. Immunoelectrophoretic pattern of fetal and newborn serum above, corresponding autoradiograph below. The latter reveals labeled γ_a in embryo serum, and labeled γ_a, γ_b, and γ_c in newborn chick serum. B. Immunoelectrophoretic pattern of 12-day-old chicken serum above, autoradiograph below. γ_a, γ_b, and γ_c are labeled. Note absence of labeling of γ globulin and β macroglobulin.

and β globulins, at least 3 proteins of slow mobility, which migrate electrophoretically with the γ-globulins. Formation of γ-globulin and β_2-macroglobulin, the major known immune globulins in other species(9), could not be shown. The relationship, if any exists, of the 3 unidentified proteins of gamma mobility to immunity has not been determined. Orlans *et al.*(10) have demonstrated, in chicken serum, 2 proteins of gamma mobility which may contain antibody, but the precipitation patterns produced in immunoelectrophoresis by their antisera differ from those described here, and cannot, therefore, be readily compared. The inability of neonatal chickens to form antibody(11) and the results of transfer experiments(12,13) indicate that these animals and embryos lack immune mechanisms present in the adult. The finding that embryos and neonatal chicks do not synthesize the major identified immune globulins is consistent with such experimental results, and is additional evidence that all of the γ-globulin found in neonatal sera is obtained from the mother *via* the yolk. The presence of proteins of slow mobility, other than γ-globulin, is of importance in evaluation of observations in free electrophoresis concerning the γ-globulin content of sera from germfree chickens(13). These preliminary findings also suggest that the prealbumin present in sera of laying hens(1) and of embryos is obtained by the embryos, from the yolk.

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Effect of Phenylalanine on Toxicity of S-(Dichlorovinyl)-L-Cysteine in the Rat and the Calf.* (27835)

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It is well documented that S-(1,2-dichlorovinyl)-L-cysteine (DCVC)(1) is very toxic for the bovine specie and for *E. coli* B. In the former it induces fatal aplastic anemia(2) while in the latter it causes inhibited and abnormal, filamentous growth(3). In contrast, the rat is much more resistant to the toxic effects of this compound.† When DCVC (about 10-20 mg/kg) is injected daily into young rats over prolonged periods, it can produce inhibition and arrest of growth, kidney

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