Association of "Lanthanum-Staining Material" with Hemagglutination by Rubella Virus[1] (34147)

P. C. TAYLOR DICKINSON,[2] TE-WEN CHANG, AND LOUIS WEINSTEIN

Infectious Disease Service of the New England Medical Center Hospitals and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

The recent isolation of rubella virus (1, 2) and the relation of human infection to fetal damage has led to intensive efforts to define the properties of this agent. Most of the studies have focused on the rubella hemagglutinating antigen which has been shown to have variable degrees of affinity for erythrocytes from sheep, goose and 24-hr-old chick sources (3). The most reliable results were obtained with erythrocytes of unfed 24-hr-old chicks; those of adult chickens are usually not agglutinated.

In this laboratory and in the work of others (4), the rubella hemagglutination reaction was only slightly sensitive to changes in pH between 6.0 and 8.2; slightly higher titers were produced in buffered borate at pH 7.0–7.2. The reaction required the presence of cations (4). Addition of EDTA to cell suspensions blocked agglutination by the virus (4). One to twofold higher titers were produced when DEAE-dextran was added to sheep red blood cell suspensions before addition of the antigen. Attempts to alter hemagglutination by treatment of erythrocytes with various enzymes have produced the following results: Prior exposure of newborn chick cells to trypsin produced nonspecific hemagglutination (5). When the erythrocytes were treated with neuraminadase, rubella hemagglutininination titers were not altered (5). Studies using density gradient centrifugation of crude antigen preparations have revealed an inhibitor of the rubella hemagglutination which was isolated and tentatively identified as a β-lipoprotein (6). These general properties of the hemagglutination reaction have been confirmed by the writers.

The characteristics of rubella hemagglutination described above bear a striking similarity to the properties of the "lanthanum-staining material" (LSM) (7) described by Lesseps (8). He examined chick embryo retina, limb bud and cardiac cells by electron microscopy and noted a lacy LSM attached to the external surface of the plasma membrane of these cells. He demonstrated that the LSM was removed by treatment of the cells with 0.001 mg/ml of phospholipase C for 10 min at 37°. The purpose of the present study was to determine if the LSM was present on the surface of chick erythrocytes and if this layer was involved in hemagglutination by rubella virus.

Methods. Erythrocytes obtained from 24-hr-old unfed chicks and adult chickens were collected in Alselver's solution and washed 3 times in dextran gelatin Veronal (DGV) buffer solution before use.

Phospholipase C (7 units/mg) was dissolved in DGV buffer and 7 dilutions of the enzyme (0.005 mg to 0.0005 mg/ml) were prepared. Equal volumes of both adult and newborn chick red cell suspensions were exposed to each concentration at 37° for 10 min. Treatment was terminated by immersing the reaction mixture in ice. The enzyme was then removed by washing the cells 3 times in DGV buffer.

Rubella hemagglutination using Tween 80–ether-treated antigen (Microbigoloical Associates) was carried out using the macro method described by Stewart (3). An equal

[1] Supported by Training Grant No. 5TIAI-276 from the Institute of Allergy and Infectious Disease, National Institutes of Health, U.S. Public Health Service.

[2] Trainee, Institute of Allergy and Infectious Disease, National Institutes of Health, U. S. Public Health Service.

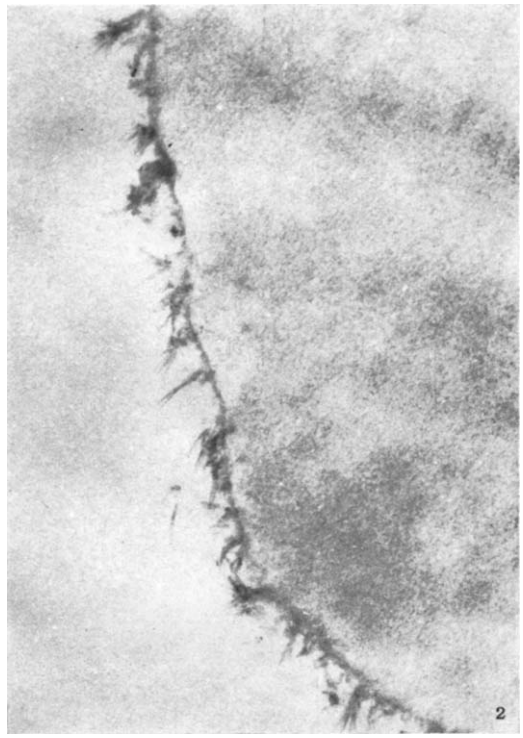

FIG. 1. Unfed 24-hr-old chick red blood cells hemagglutinated with rubella antigen and stained with lanthanum ions; 5 mm = 300 Å.

volume of enzyme-treated (each concentration) or untreated erythrocytes was added to each dilution of antigen, and titers were determined after incubation at 4° overnight.

All cells were fixed and stained with lanthanum ions by the method of Lesseps (8). The cells were then washed 3 times in phosphate buffered saline to remove excess stain, embedded in Aralite 502 (9) and thin sections were examined, using an RCA-EMU-3G microscope.

Results. The agglutinin titers for untreated 24-hr-old chick erythrocytes ranged from 1:8 to 1:32. The addition of a concentration of 0.0025 mg/ml of phospholipase C produced hemolysis. When 0.001 mg/ml of the enzyme was added, the rubella antigen failed to produce agglutination but the cells remained intact. Untreated adult chick cells and control chick cells without antigen present did not agglutinate.

Electron microscopy of untreated newborn chick erythrocytes which had been agglutinated by rubella antigen revealed a thick lacy structure covering the outer surface of the plasma membrane and bridges between adjacent cells (Fig. 1). This had the same appearance as the previously described lanthanum staining material (7). Control cells which had not been treated with rubella antigen also had this same appearance. When newborn cells, treated with 0.001 mg/ ml of phospholipase C were examined, it was noted that most of this material had been stripped away from the outer surface of the normal plasma membrane while the intracellular space retained its hemoglobin (Fig. 2). Cells which had been completely lysed by the enzyme retained the plasma membrane structure but had completely lost the lacy structure on the outer surface. These cells were not agglutinated by rubella virus. In untreated red blood cells of grown chickens, the thickness of this layer was markedly reduced and the lacy appearance characteristic

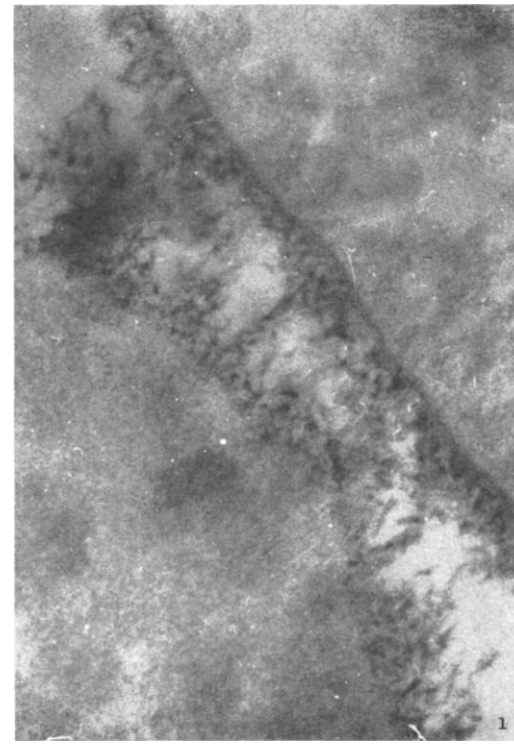

FIG. 2. Unfed 24-hr-old chick red blood cell treated with 0.001 mg/ml of phospholipase C; 5 mm = Å.

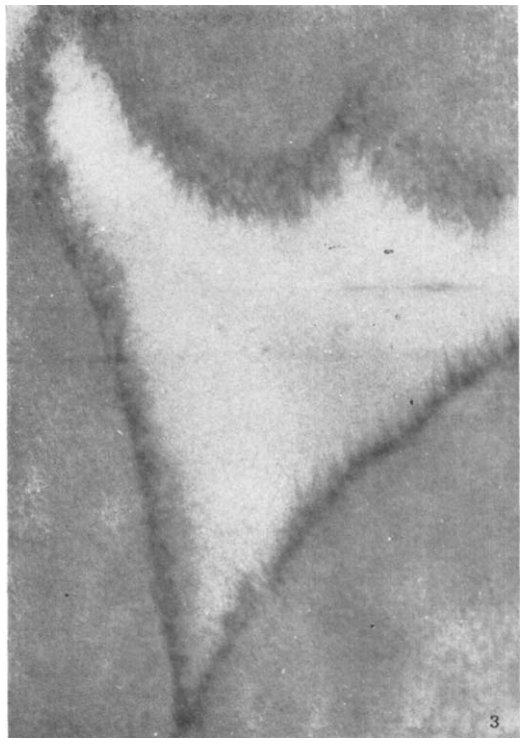

FIG. 3. Adult chicken red blood cells stained with lanthanum ions; 5 mm = 300 Å.

of the erythrocytes of newborn chicks was not apparent (Fig. 3).

Discussion. The red blood cells of newborn chicks appear to have a thick layer of material external to the previously recognized triphasic plasma membrane on their surface. This layer has the same properties as the lanthanum-staining material demonstrated on other chick embryo cells (8). Minute quantities of phospholipase C remove the LSM but do not destroy the integrity of the plasma membrane. Adult chicken cells possess a much thinner layer of LSM which has lost its lacy quality. The lanthanum-staining material appears to be essential for agglutination of 24-hr-old chick erythrocytes by rubella virus. Recent studies demonstrated an inhibitor of rubella hemagglutination in crude preparations of antigen. It is perhaps more than coincidence that this inhibitor, thought to be a β-lipoprotein, can be destroyed by phospholipase C (6).

Summary. Erythrocytes from 24-hr-old chicks have, on the external surface of the plasma membrane, a structure which appears to possess all of the characteristics of previously described lanthanum-staining material. Removal of this layer by treatment with very low concentrations of phospholipase C results in failure of the cells to be agglutinated by the virus.

1. Parkman, P. D., Buescher, E. L., and Artenstein, M. S., Proc. Soc. Exptl. Biol. Med. **111**, 225 (1962).

2. Weller, T. H. and Neva, F. A., Proc. Soc. Exptl. Biol. Med. **111**, 215 (1962).

3. Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, R. D., Hamilton, J. P., and Meyer, H. M., Jr., New Engl. J. Med. **276**, 554 (1967).

4. Furukawa, T., Plotkin, S. A., Sedwick, W. D., and Profeta, M. L., Proc. Soc. Exptl. Biol. Med. **126**, 745 (1967).

5. Furukawa, T., Plotkin, S., Sedwick, D., and Profeta, M. Nature **215**, 172 (1967).

6. Sedwick, W. D., Furukawa, T., Plotkin, S. A., Paper presented: Bacteriol. Proc. **180** (1968).

7. Doggneweiler, C. F. and Frenk, S., Proc. Natl. Acad. Sci. U.S. **53**, 425 (1965).

8. Lesseps, R. J., J. Cell Biol. **34**, 173 (1967).

9. Luft, J. H., J. Biophys. Biochem. Cytol. **9**, 409 (1961).

Received April 21, 1969. P.S.E.B.M., 1969, Vol. 132.