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Presence of Granules Resembling Elementary Bodies in Yolk Cells of Normal Eggs.

ALWIN M. PAPPENHEIMER, ELEANORA MOLLOY, AND HARRY M. ROSE.

*From the Department of Pathology and the Department of Medicine,
College of Physicians and Surgeons, Columbia University, New York City.*

Blood from a case of anemia associated with the presence in the red blood cells of unidentified siderophilic granules¹ was inoculated onto the chorio-allantoic membranes and into the yolk sac of developing chick embryos. Sections were prepared from the membranes and, in Giemsa-stained preparations, minute purplish blue granules were found in great numbers in the cytoplasm of the yolk cells. In order to reach a conclusion as to their possible identity with the intra-erythrocytic bodies, control eggs were inoculated with saline, normal blood, influenza virus, and other ma-

terials; and finally, uninoculated eggs in various stages of development were examined. It was surprising to find in all eggs, treated or untreated, after the 11th or 12th day, increasing numbers of these granules (Fig. 1). Because of their close resemblance to described elementary bodies of certain viruses, and because we have found no reference to their occurrence in uninoculated eggs, we desire to give a brief account of our observations.

Paraffin sections of the membranes were prepared from more than 200 eggs fixed in 10% formalin, Zenker's fluid without acetic acid and

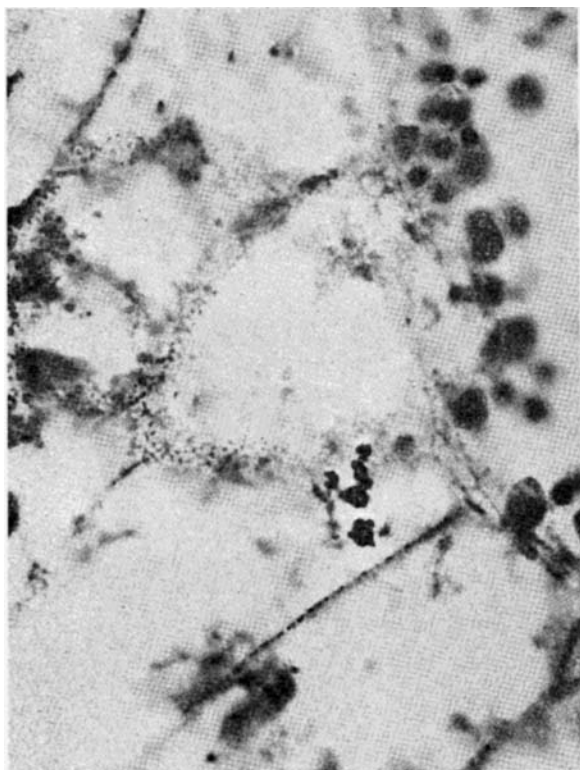


FIG. 1.

Yolk sac. Giemsa stain. $\times 1050$. Egg inoculated on 6th day of incubation with blood from patient K. Taken for examination on 14th day.

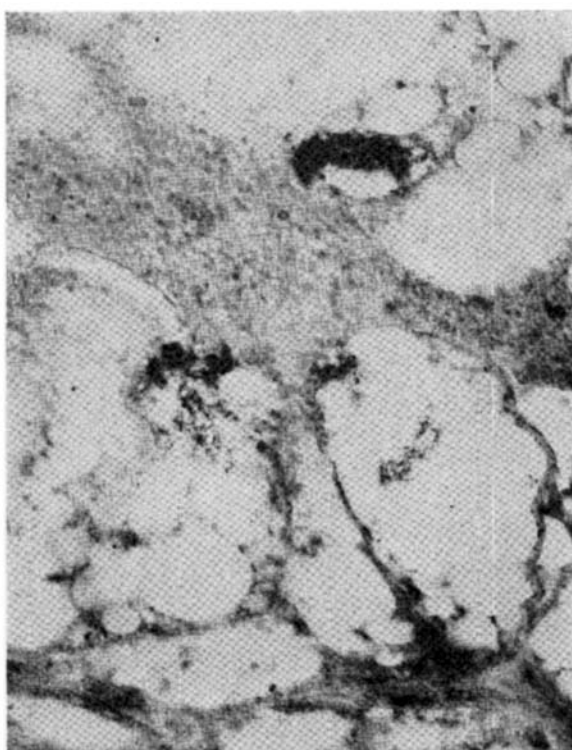


FIG. 2.

Yolk sac, normal uninoculated egg. 17 days incubation. Giemsa stain. $\times 1050$.

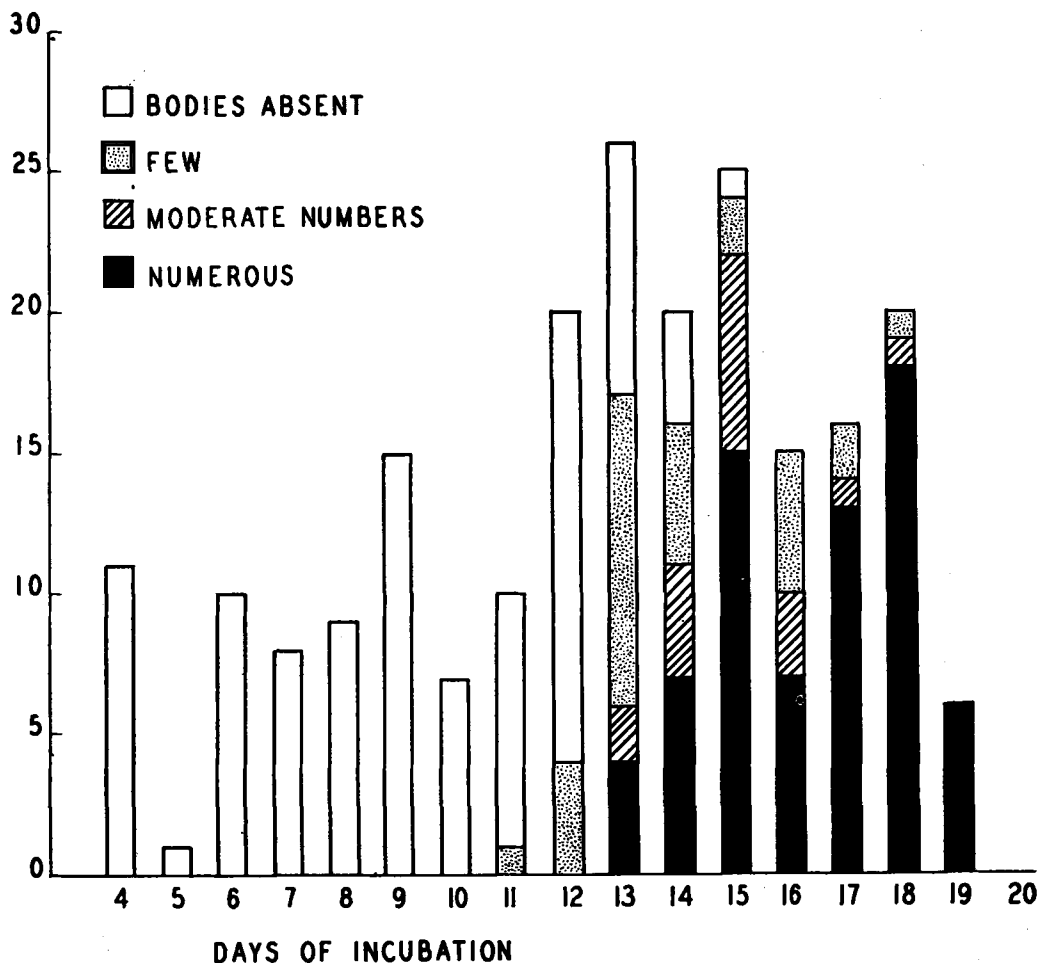
NO OF EGGS.
EXAMINED

CHART I.

Bouin's fluid.* They were stained with Giemsa, eosin-methylene blue, Noble's stain, and by Nyka's rickettsial stain,² and the bodies were readily demonstrable by all these methods. The bodies were found within the yolk cells as scattered individuals, or as large agglomerations. Their size varied from a fraction of a micron up to a maximum of 1

or 2 μ . The staining also varied somewhat, the minute forms taking a reddish purple stain with Giemsa and the larger forms a more intense blue. Diploid forms were repeatedly seen and, occasionally, short delicate rod shapes. The bodies were often aligned in a single row along the wall of the cells; commonly large colonial aggregations occupied the shreddy protoplasmic processes which projected from the cell wall. (Fig. 1, 2, 3). They were rarely found among the extra-cellular yolk granules, and among the developing blood cells in the sinuses. It is apparent from the accompanying chart that the bodies first make their appearance about the 11th or 12th day of incubation, and from this time on are found

* The eggs used in this study were White Leghorns, obtained from the Shamrock Poultry Farms, New Brunswick, N. J.

¹ Pappenheimer, A. M., Thompson, W. P., Parker, D., and Smith, K. E., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 145.

² Nyka, W., *J. Path. and Bact.*, 1944, **56**, 264.

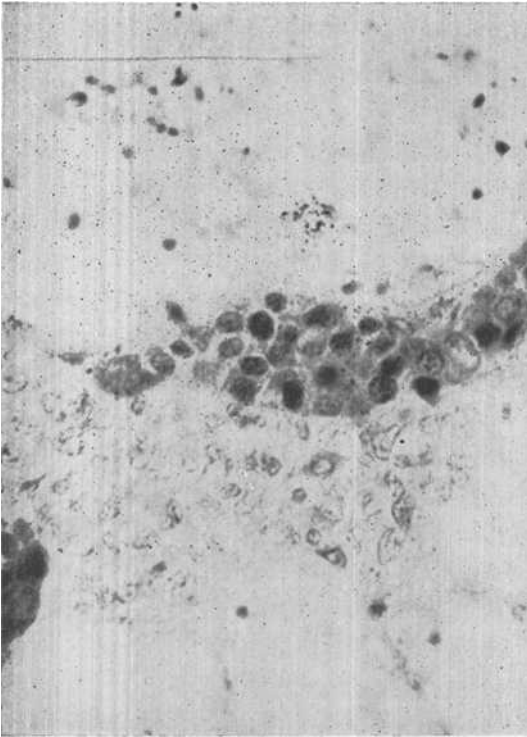


FIG. 3.
Yolk sac, normal uninoculated egg. 18 days' incubation. Nyka's fuchsin-methyl violet stain. $\times 1050$.

in increasing numbers up to the 19th day.

That the bodies are due to staining artefacts

seems highly improbable, since they may be demonstrated after different methods of fixation, with different staining techniques, and have a well-defined morphology and sharp outline. Their absence in the yolk cells before the 10th day is a further argument against their being artefacts. There remain two possible interpretations of their nature:

1. They are cytoplasmic constituents of normal yolk cells, appearing in the course of their evolution. Against this viewpoint is the fact that they are not present in all cells and indeed, during the first few days after their appearance, may be scant in number and require prolonged search for their detection.

2. They are elementary virus bodies or psittacosis-like micro-organisms, present as harmless commensals in the developing yolk cells. The finding of diploid forms and the progressive increase in the numbers of the bodies suggests active multiplication. Further experiments are obviously indicated before drawing conclusions as to the nature of these bodies.

Since the submission of this proof, identical bodies have been found in the yolk cells of eggs from Barred Plymouth Rocks and Rhode Island Reds sent to us from the Storrs Agricultural Experiment Station through the courtesy of Dr. Erwin Jungherr.

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Adaptation of Influenza Virus to Heat.*

MARION JONES. (Introduced by Wm. M. Hale.)

From the Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City, Iowa.

Bacteria have been adapted many times to growth or survival under a number of physical conditions, including heat, which are entirely foreign to any environmental circumstances

encountered in normal multiplication. Due to numerous technical difficulties, very few similar studies with viruses have been carried out.

Armstrong¹ was able to select a heat-resistant strain of vaccinia virus by passing samples of virus which withstood the longest storage periods at 37°C. At the end of the 63rd transfer, the virus withstood that temp-

* This investigation was aided in part by the Commission on Influenza Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Washington, D.C.

¹ Armstrong, Charles, *Pub. Health Rep.*, 1929, 44, 1183.