16343

Cultivation of *Rickettsia prowazeki* in Dead Chick Embryos.

ESTHER RABINOWITZ, M. ASCHNER, AND N. GROSSOWICZ. (Introduced by L. Olitzki.)
From the Department of Hygiene and Bacteriology, Hebrew University.

The criterion commonly used for the determination of the state of life or death of an organism is the heartbeat. Chick embryos in which the heart has ceased to function must be regarded as dead, and this assumption is supported by the fact that such embryos are indeed incapable of further development.

However, an egg containing a dead embryo is not necessarily devoid of living cells. It is known that the cells of an organism may survive after death for varying periods, depending on the external condition and on the type of tissue from which the cells were derived. Individual cells in fully developed tissues generally survive no more than a few hours if they remain in contact with the dead organism. However, in an embryo, in which cells are probably less interdependent, conditions need not be, and indeed are not, the same.

It has been demonstrated by Bucciante[1] that dead chick embryos may contain living cells for as long as 31 days after cessation of the heart beat, if the egg be maintained at 20°C after the death of the embryo; survival times at lower temperatures were even longer, up to 47 days.

It occurred to us that eggs containing surviving rather than developing tissue might constitute a favorable medium for the cultivation of *Rickettsia prowazeki* and related organisms.

According to Zinsser and Schoenbach[2] the rickettsiae develop best in cells with a low metabolic rate, and such a state may be assumed to occur in cells which survive without development for extended periods. However, the question was whether the cells would survive sufficiently long at temperatures high enough for the development of rickettsiae.

Grodzinski,[3] who examined the survival at 38°C of aorta tissue from dead chick embryos, reported a maximum survival time of 3 hours, which is, of course, insufficient for the cultivation of rickettsiae. However, this author tested only one type of tissue and worked with relatively well developed embryos, the youngest being 7 days old.

We tried using embryos which had been killed by chilling, after only 3 days development, and found that living cells could still be demonstrated in such material after much longer periods, even if the eggs had been maintained during this time at 37°C.

The following two cases may serve as illustrations: An egg containing a 3-day-old embryo was chilled at 4°C for 24 hours, and, after storage for 4 days at room temperature, was incubated for 10 days at 37°C. When the egg was opened after this period it contained a dead embryo which had not developed beyond its third day, yet living cells, mostly of epithelial character, grew out when fragments of the embryo or of its membrane were cultured in plasma clots.

In another case living cells were demonstrated by the same technique in an embryo which had been kept after death at room temperature for 7 days, and then for 16 days at 37°C. Results were practically the same in cases in which the embryos died spontaneously during the first 3 days of incubation as in those in which they were killed by exposure to cold.

R. prowazeki was inoculated into the yolk sac of eggs containing dead embryos and these were incubated for varying periods of time at 37°C. As expected, the rickettsiae multiplied abundantly under these conditions. It

[1] Bucciante, L., *Arch. exp. Zellforsch.*, 1931, **11**, 397.

[2] Zinsser, H., and Schoenbach, E. B., *J. Exp. Med.*, 1937, **66**, 207.

[3] Grodzinski, Z., *Arch. exp. Zellforsch.*, 1932, **12**, 587.

is difficult quantitatively to compare the growth of rickettsiae in dead embryos with that in living ones, as several factors obviously influence the final result. The maximum number of rickettsiae obtainable per egg is perhaps greater in eggs containing living embryos, since their yolk sacs are more extensive than those of eggs which have developed for only 3 days. However, the concentration of rickettsiae per cell in the infected cells of dead embryos may surpass that reached in living ones.

The growth period for rickettsiae in dead embryos may easily be extended to 16 days, or double the time customary in work with living embryos. Therefore even minute inocula have a chance to multiply significantly. This was determined in the following manner: 3-day-old dead embryos and 7-day-old living embryos were inoculated in parallel series with decreasing amounts of the same suspension of rickettsiae. Inocula too small to yield a positive smear after the usual 7-days' incubation in the living embryos yielded smears containing numerous rickettsiae after 14 days' incubation in the dead embryos.

Several advantages are inherent in our method. Eggs have to be incubated prior to use for only 3 days, and may then be stored for sometime at room temperature like ordinary culture media. This fact is especially convenient in field work, in which eggs may be inoculated on the spot and thereafter transported to the laboratory without particular regard to correct incubation temperature, humidity, or protection from vibration during transport, as is necessary when dealing with living eggs. Furthermore, this method is economical, since the percentage of eggs which have to be discarded on account of premature death of the embryo is, of course, considerably reduced.

Summary. 1. Chick embryos which were killed by chilling on the 3rd day of development and were thereafter maintained at 37°C for 16 days still contained living cells.

2. *Rickettsia prowazeki* multiplied abundantly in dead chick embryos which contained surviving cells.

3. The significance of these findings and some advantages inherent in this method are discussed.

16344

Sectioning Techniques for Electron Microscopy Using a Conventional Microtome.

DANIEL C. PEASE AND RICHARD F. BAKER.

From the Departments of Anatomy and Experimental Medicine, School of Medicine, The University of Southern California, Los Angeles, Calif.

Sectioning biological material for use with the electron microscope poses special problems. Useful thicknesses can be measured only in fractions of a micron so that cutting becomes a major difficulty. Sections have to be examined *in vacuo,* so that the mode of drying is a matter for serious consideration.

The authors know of only 3 prior attempts to adapt more or less conventional histological techniques to the preparation of specimens for the electron microscope. Richards, Anderson and Hance[1] took embedded material and faced the block in a standard microtome. They then changed the angle of the block and cut wedges which they hoped would taper into the fractional micron range. To a certain extent they were successful, but apparently were unable to find a completely satisfactory solution to embedding and mounting problems. Von Ardenne[2] employed essentially the same principle. Sjöstrand[3] has also attempted

[1] Richards, G. A., Anderson, T. F., and Hance, R. T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 148.