Cytoplasmic Changes in Primary Neural Induction

It has recently been reported that at the time of primary neural induction a band of nuclei appears in the ectoderm anterior to Hensen's node[1]. Because there have been very few studies of this region by electron microscopy[2], a further attempt has been made to clarify the ultrastructural aspects of primary neural induction.

White leghorn chick embryos were incubated at 37.5 °C, dissected off the yolk in PANNETT and COMPTON[3] saline, and mounted on glass rings as described by NEW[4].

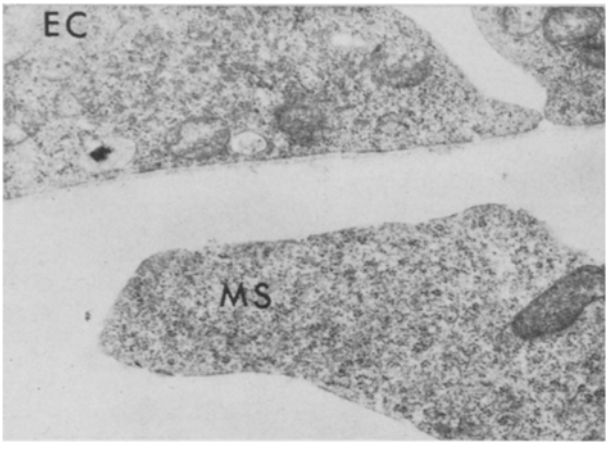

Fig. 1. Transverse section anterior to Hensen's node of the normal stage 4 chick embryo. The cell organelles are distributed throughout the cytoplasm. EC, ectoderm; MS, mesoderm. ×22,500.

Those embryos at stage-5 (10 embryos) and stage 4 (10 embryos) were then fixed in KARNOVSKY's fluid[5] (pH 7.2–7.4) followed by Cacodylate buffer[6] (pH 7.4), osmium tetroxide solution[7] (pH 7.4) and dehydrated in a graded series of ethanol/water and embedded in Araldite[8]. They were then sectioned for electron microscopy, stained with lead citrate[9], and examined with a Siemens Elmiskop IB (80 kV) transmission electron microscope. A further 10 embryos (stage-5) were fixed in ovo with KARNOVSKY's fluid to be compared with the specimens mounted by New Culture.

In the stage 4 ectodermal and mesodermal cells, organelles are scattered throughout the cytoplasm. Mitochondria, ribosomes, granular and agranular reticulum, lipid drops, and yolk granules are spread throughout each cell (Figure 1). A distinct basement membrane is present lining the ventral border of the ectoderm layer.

In the stage-5 embryo sectioned in the region where the band of nuclei appears by light microscopy, the organelles are similarly scattered throughout the cytoplasm of the

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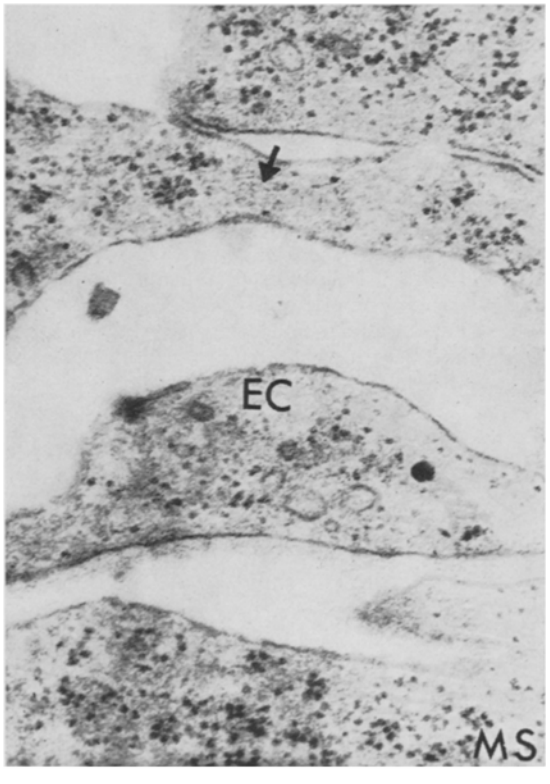

Fig. 2. A transverse section of the normal stage-5 chick embryo anterior to Hensen's node. Cell organelles are absent (arrow) in both the ectoderm (EC) and mesoderm (MS) cells. ×60,000.

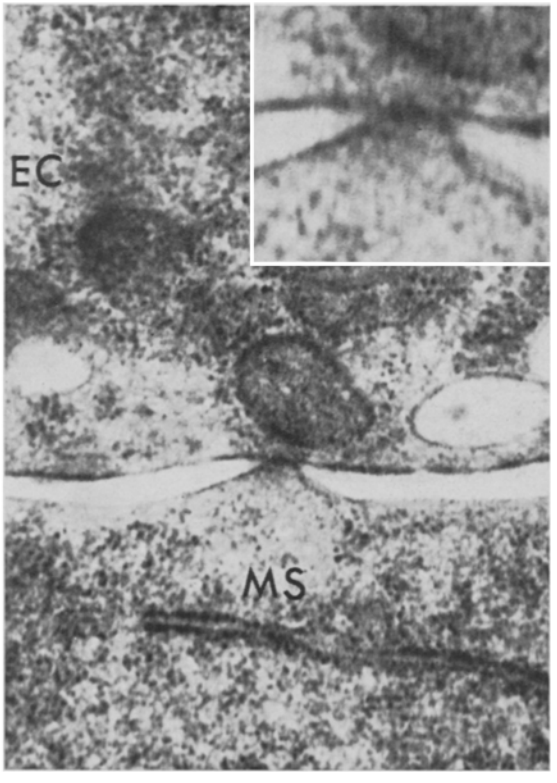

Fig. 3. An area adjacent to the region in Figure 2. The basement membrane is compressed between a mesoderm cell (MS) and an ectoderm cell (EC). × 52,000, inset ×130,000.

ectoderm and mesoderm cells except in the area of the basement membrane. The cytoplasm of both types of cells is lacking organelles where these cells are in close proximity to one another in the area of the basement membrane (Figure 2). The region is notable for the absence of ribosomes. Associated with these areas are mesoderm cells which have compressed the basement membrane against the ectoderm cells (Figure 3).

Similar observations were also made in the stage-5 in ovo specimens. Cytoplasmic changes were evident in the region where the band of nuclei appears by light microscopy. Mesoderm cells have compressed the basement membrane against the ectoderm layer and ribosome free areas are present in this region.

These observations suggest that primary neural induction in the chick embryo is associated with cellular communication between ectoderm and mesoderm cells. Furthermore, the cytoplasmic changes in the mesoderm emphasize the close interaction between ectoderm and mesoderm during induction.

Résumé. Exposé d'une étude ultrastructurale de l'induction neurale primaire chez l'embryon de poulet. Entre l'étape 4 et l'étape-5, les cellules mésodermiques compriment la membrane basale contre les cellules ectodermiques. Le cytoplasme des cellules mésodermiques et ectodermiques se libère d'organelles, spécialement de ribosomes, dans la région adjacente à la membrane basale. Ce fait suggère l'existence d'une communication cellulaire entre les cellules ectodermiques et mésodermiques, et ce processus peut être responsable de l'induction neurale primaire.

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EPSTEIN-BARR Virus-Binding Receptor on the Surface of Chronic Lymphocytic Leukaemic Lymphocytes

The nonpermissive or partially permissive interaction of the EPSTEIN-BARR virus (EBV) with the lymphoid cells has been clearly demonstrated, but other cells susceptible to the viral replication have not yet been found, and so the question arises whether the T or the B lymphocytes, or both, are susceptible to the EBV infection. JONDAL and KLEIN[1] have studied this problem and found that the B lymphocytes originating from healthy donors were the target cells and all EBV-carrying continuous human lymphoblastoid cell lines had receptors characteristic for B lymphocytes (the presence of surface-bound immunoglobulin molecules). In contrast, established cell lines of known T cell origin did not carry the EBV genome. These facts clearly indicate that the EBV is a B cell tropic virus. Experimental results show the occurence of B cell proliferation in chronic lymphocytic leukaemia (CLL) or, in other words, the CLL lymphocytes have characteristic surface markers of B cells[2,3]. For this reason it is of interest to study the susceptibility of CLL lymphocytes for the EBV infection, and it seems especially important to establish the presence or absence an EBV-receptor on the surface of CLL lymphocytes. In our experiments we used the special rosette test described by JONDAL and KLEIN, with only a slight modification. The virus receptors on the surface of the sensitive lymphocytes combine with the viral envelope materials accumulated in the membranes of membrane antigen positive cells. This finding indicates that the susceptible cells have to bind to the EBV-producing cells in the same way in which the EBV acts on the target cells. P3HR-1 is one of the best virus-producing lines, containing 'whole ring' membrane antigen positive cells.

Materials and methods. Purified CLL lymphocytes were mixed with EBV-producing P3HR-1 cells originating from Burkitt lymphoma at a ratio of 20 to 1. The cells were suspended in 0.3 ml PBS and incubated at 4 °C for 1 h. Then the suspension was dropped into slides, air dried, fixed in cold acetone-methanol (1:1) and stained with FITC conjugated anti-EBV-VCA-MA (virus capsid antigen and membrane antigen) immunsera. It has been shown previously that all VCA positive cells are also MA positive in EBV carrier cultures[4]. Samples were taken

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Binding of CLL lymphocytes to EB virus producing cells in the P3HR-1 line

CLL cases	Presence of surface bound immunoglobulins on the CLL cells[a]	VCA positive cells (%)	Rosette forming cells (%)	VCA positive cells forming rosettes (%)	VCA negative cells forming rosettes (%)
1	positive	2.5	2.0	90	0
2	positive	2.5	2.0	90	0
3	positive	3.0	3.0	95	0
4	positive	1.5	1.5	90	0
5	positive	3.0	3.0	90	0
6	positive	2.5	2.0	80	0

[a] Detected by membrane immunofluorescence with FITC labelled anti human IgM conjugate (Hyland).