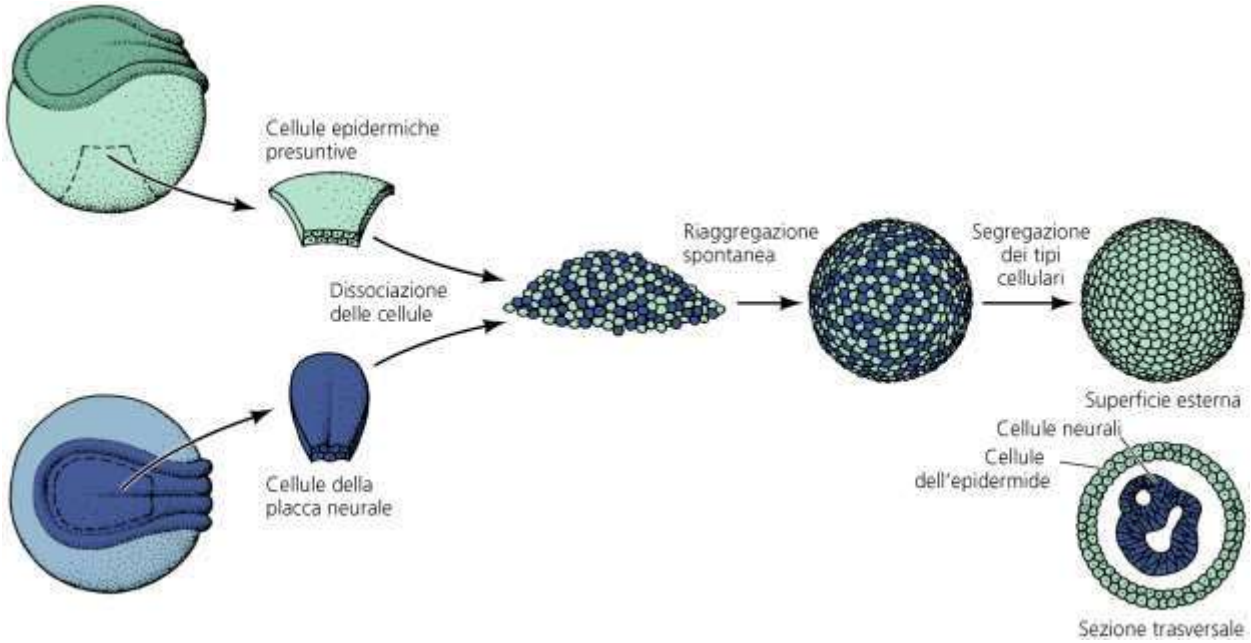
MORPHOGENESIS AND CELL ADHESION

There are two major types of cells arrangement in the embryo: epithelial cells, which is tightly connected to one another in sheets or tubes, and **mesenchymal cells,** which are unconnected to one another which operate as independent unit. Morphogenesis is brought about through a limited repertoire of variation in cellular process within these two type of arrangements: (1) the direction and number of cell divisions; (2) cell shape change; (3) cell movement; (4) cell growth; (5) cell death and (6) change in composition of cell membrane.

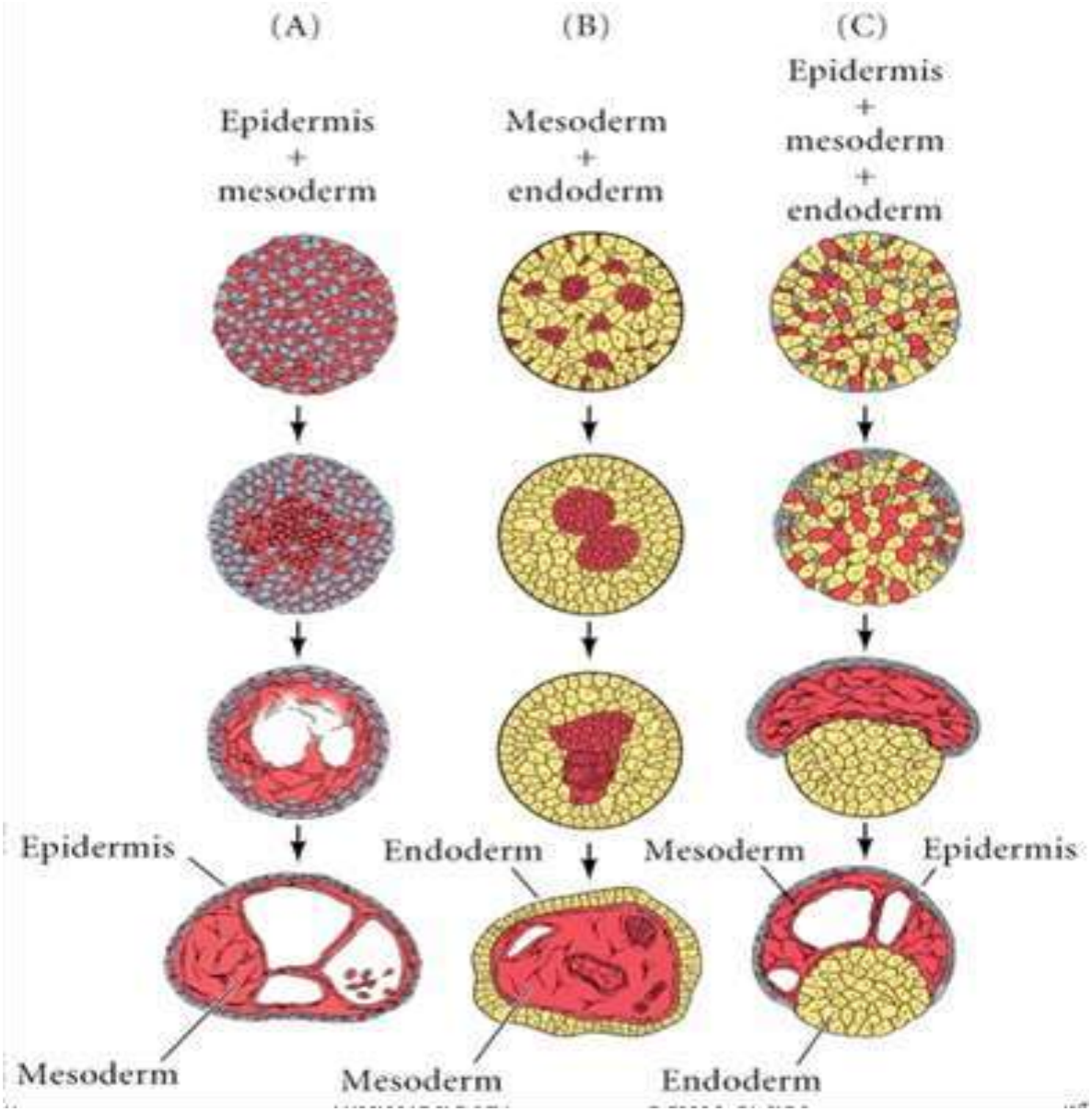
Differential cell affinity

The cell surface looks pretty much the same in all cell types, and many early investigators thought that the cell surface was not even a living part of the cell. We now know that each type of cell surface and that some of these differences are responsible for forming the structure of the tissues and organs during development. Fertilization and early embryonic development observations by E.E. Just (1939) suggested that the cell membrane differed among cell type but the modern analysis of morphogenesis began with experiment of Townes and Holtfreter in 1955. Taking advantage of discovery the amphibian tissue become dissociated into single cells when placed in alkaline solution, they prepare single cell suspension from each of the three germ layer of amphibian soon after the neural tube has formed. Two or more of these single cell suspensions could be combined in various way, and when the pH was normalized, the cell adhered to one another forming aggregation on agar coated petri dishes. By using embryo from species having cells of different size and color, Townes and Holtfreter are able to follow the behavior of the recombined cells.



Reaggregation of cells from amphibian neurulae; Presumptive epidermal cells from pigmented embryo and neural plate cells from unpigmented embryos are dissociated and mixed together. The cells reaggregate so that one type (here, the presumptive epidermis) covers the other.

The **result of their experiment** was striking. First they found that reaggregated cells become spatially segregated. That is instead of two cell types remaining mix each cells type sorts out into own region. Thus, when ectodermal and mesodermal cells are brought together to form a mixed aggregates, the epidermal cell moves periphery of the aggregates and mesoderm move to inside. In no case do the recombined cells remain randomly mixed and in most cases one tissue type completely envelops the other.



Sorting out reconstruction of spatial relationshipin aggregates of embryonic amphibian cells.

Second the researchers found that the final positions of the reaggregated cells reflect their embryonic positions. The mesoderm migrates centrally with respect to the epidermis, adhering to the inner epidermal surface. The mesodermal also migrate centrally with respect to the gut or endoderm. However when the three germ layers are mixed together the endoderm separate from ectoderm and mesoderm and is then envelop by them. In its final configuration the ectoderm is on periphery the endoderm is internal and mesoderm is lies in between them. Holtfreter interpreted this finding in term of **selective affinity**. The inner surface of ectoderm has a positive affinity for mesodermal cells and a negative affinity for the endoderm while the mesoderm has positive affinities for both ectodermal and endodermal cells. Mimicry of normal embryonic structure by cell aggregates is also seen in the recombination of the epidermis and neural plate cells. The presumptive epidermal cells migrate to the periphery as before the neural plate migrate inward forming a structure reminiscent of the neural tube. When axial mesoderm cells are added to the suspension of presumptive epidermal layer, a central located neural tissue and a layer of mesodermal tissue between them are formed. Somehow the cells are able to sort out into their embryonic positions.

The third conclusion of Holtfreter and colleagues was the selective affinities change during development. This should be expected because embryonic cells do not retain a single stable relationship with other cell types. For development to occur, cells must interact differently with other cell population at specific time. Such changes in cell affinity are extremely important in the processes of morphogenesis.

The reconstruction of aggregates from cells of later embryos of birds and mammals was accomplished by use of the protease trypsin to dissociate the cells from one another. When resulting single cells were mixed together in a flask and swirled so that the shear force would break any non-specific adhesion the cell sorted them out according to their cell type. In so doing they reconstructed the organization of real tissue show the reconstruction of skin tissue from a 15 day embryonic mouse. The skin cells are separated by proteolytic enzyme and then aggregate in rotatory culture. The epidermal cells aggregate migrate to the periphery and the dermal cell migrate to the periphery and dermal cells migrate toward the center. In 72 hours the epidermis has been reconstituted a keratin layer has formed and interaction between these tissues from individual cells is called **histotypic aggregation.**

CADHERINS AND CELL ADHESION:

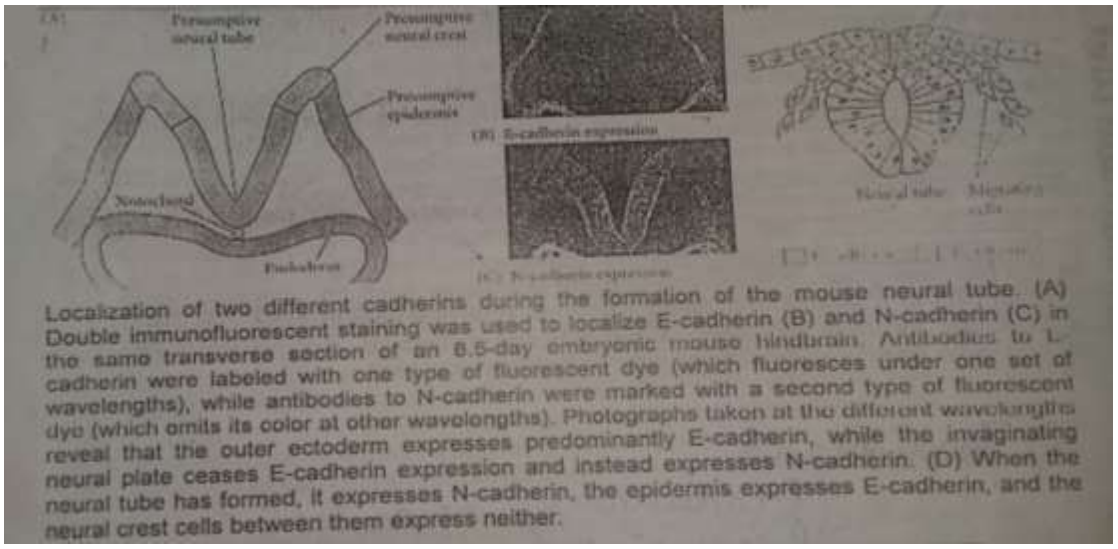
Recent evidence shows that boundaries between tissues can indeed be created both by (1) different cell types having different type of cell adhesion molecules and (2) different cell types having different cell type‘s haing different type of cell adhesion molecules. There are several classes of molecule that can mediate cell adhesion. The major cell adhesion molecule appears to be **cadherins**. As their name suggest, they are calcium dependent adhesion molecules. Cadherins are critical for establishing and maintaining intercellular connection and they appear to be crutial to the spatial segregation of cell type and to the organization of animal form. Cadherin interact with other cadherins on adjacent cells and they anchored into the cell by a complex of protein called catenin.

The catenin complex forms the classic adherent junctions that connect epithelial cell together. Moreover, since the catenins bind to the actin cytoskeleton of the cells together. Moreover since the catenins bind to the actin cytoskeleton of the cell, they integrate the epithelial cell together into mechanical unit.

In vertebrate embryo several major cadherin class has been identified

i) **E- cadherin** epithelial cadherin also called uvomorulin and L-CAM is expressed on early mammalian embryonic cells even at the 1-cell stage. Later this molecule is restricted to epithelial tissue of embryo and adults.

ii) **P-cadherin** (placental cadherin) appear to be expressed primarily on the trophoblast cell (those placental cells of mammalian embryo that contact the uterine wall) and on the uterine wall epithelium. It is possible that P-cadherin facilitates the connection of embryo to the uterus, since p cadherin ion the uterus cell is seen to contact p-cadherin on the trophoblast cell of mouse embryos.



Localization of two different cadherins during the formation of the mouse neural tube. (A) Double immunofluorescent staining was used to localize E-cadherin (B) and N-cadherin (C) in the same transverse section of an 8.5-day embryonic mouse hindbrain. Antibodies to E-cadherin were labeled with one type of fluorescent dye (which fluoresces under one set of wavelengths), while antibodies to N-cadherin were marked with a second type of fluorescent dye (which omits its color at other wavelengths). Photographs taken at the different wavelengths reveal that the outer ectoderm expresses predominantly E-cadherin, while the invaginating neural plate ceases E-cadherin expression and instead expresses N-cadherin. (D) When the neural tube has formed, it expresses N-cadherin, the epidermis expresses E-cadherin, and the neural crest cells between them express neither.

iii) **N-cadherin** (neural cadherin) is first seen in mesodermal cell in the gastrulating embryo as they lose their e-cadherin expression . it is also called highly expressed on cells on developing central nervous system

iv) **EP-cadherin** (C-caherin) has found to be critical for maintain adhesion between the blastoderm of the Xenopus blastula and is required for normal movement of gastrulation.

v) **Protocadherin** are calcium dependent adhesion protein that different from classic cadherins in that the lack connection to cytoskeleton to catenins. Protocaherins have been found to be very important in separating the notochord from the other mesodermal tissue during Xenopus gastrulation.

Cadherin join cell together by binding to the same type of cadherin on another cell. Thus cell with E-cadherin stick best to other cells with E-caherin and they will sort out cell with N-cadherin in their membrane. This pattern is called **homophillic binding**. Cell expressing N-cadherin readily sort out from N-cadherin negative cell in vitro and univalent (fab) antibodies against cadherin will convert a three dimensional histotypic aggregate of cell into single layer of cell. Moreover when activated E-cadherin genes are added to and expressed in cultured mouse fibroblast, E-cadherin is seen on their cell surface and the treated fibroblast become tightly connected to one another. In the fact these cell begin acting like epithelial cell. The sorting out of cell can be explained by amount and types of cadherin on their cell surface. Fibroblasts made to express E-cadherin adhere to other E-cadherin bearing fibroblast while fibroblast made to express P-cadherin stick to other fibroblast expressing P-cadherin.

These adhesion patterns may have important consequence in the embryo. In the gastrula of frog Xenopus the neural tube expresses N-cadherin while the epidermis expresses E-cadherin. Normally these two tissues separate from each other such that the neural tube is inside the body and the epidermis cover the body. If the epidermis is experimentally manipulated to remove its E-cadherin the epidermal epithelium cannot hold together. If the epidermis is made to express N-cadherin or if the neural cells are made to lose it, the neural tube will not separate from the epidermis.

During development the cadherins often work withother adhesion systems. For instance one of the most critical times in the mammals life is when the embryo is passing through the uterus. If development is continues the embryo must adhere to the uterus and embedded itself to the uterine wall. That‘s why the first differentiation event in the mammalian development distinguished the **trophoblast cell** from **inner cell mass**. This process occurs as the embryo travel down from the upper region of the oviduct on its way to the uterus. The trophoblast cell endowed with several adhesion molecules to anchored the embryo to uterine wall. First they contain both E-cadherin and P-cadherin and these cadherin recognize similar cadherine on uterine cell. Second they have receptors for the collagen and heparin sulphate glycoproteine of the uterine wall. Third the trphoblast cell is also have modified glycosyltransferase enzyme that extend out the transferase membrane and can bind to the specific carbohydrate residues on uterine glycoprotein. For something as important as implantation of the mammalian embryo it is not surprising that several cell adhesion systems appear to be working together.