Meetings

Epithelial-Mesenchymal Interactions

A crucial problem in biology refers to the basic mechanisms by which cells of the same genotype eventually develop into different phenotypes. Since the classic work on primary induction by Spemann, considerable data has been gathered suggesting that the development of the morphogenetic and phenotypic characteristics of epithelial cells is, at least in part, the result of interactions of these cells with their surrounding mesenchyma. The 18th Hahnemann Symposium, held in Philadelphia 10–12 April 1967, was devoted to the subject of epithelial-mesenchymal interactions. Eighteen papers dealt with various aspects of cell interactions—cellular differentiation, morphogenesis, cell locomotion, intercellular contacts, origin of symmetry, control of mitotic activity, preservation of epithelial specificity, lymphoid differentiation, and tumor induction.

Michael Abercrombie (University College, London) discussed interactions between epithelial and mesenchymal cells as they influence the locomotion of either cell type. Contacts between moving epithelial or mesenchymal cells are made by means of ruffled membranes on one or both colliding cells. It is not known whether these ruffled membranes are locomotory organs or just the expression of locomotive activity of a cell. Both, epithelial and mesenchymal cells, when moving in vitro, show the phenomenon of contact inhibition of movement when they collide with other cells of their own kind. This process is accompanied by paralysis of the ruffled membranes and mutual adhesion of the cells. Epithelial cells seem to differ in contact behavior from mesenchymal cells in that they adhere together by their lateral edges, thus forming a coherent sheet or cord. When epithelial cells collide with mesenchymal cells, on a plane surface in vitro, the same contact inhibition phenomenon occurs, although the adhesion of the cells to each other and the inhibition of the ruffled membranes is less marked than when homologous cells collide. However, in some combinations of cell types, mesenchymal cells may collide and insert themselves between the cells of an epithelial sheet and move rather freely.

Elizabeth D. Hay (Harvard University) presented an electron microscopic study on the development of intercellular contacts during the early stages of chick embryos. Such intercellular contacts seem to be present in all embryonic tissues (epiblast, hypoblast, and mesoblast). Since an epithelium may be defined as an aggregation of cells in contiguity, one may reach the conclusion that during early stages of development all embryonic tissues are epithelia. Primary mesenchyma, derived from the epiblast, will invariably produce permanent epithelia (endothelium, mesothelium, renal epithelium) while somites, which originally form an epithelium, will eventually disband. On the other hand, secondary mesenchyma will never form cell aggregates but rather give origin to the muscle, pigment, and connective tissue cells. In reference to epithelial-mesenchymal interactions, Hay noted that tight contacts of unlike tissues only occur during the events of primary induction which is renowned for its lack of specificity. On the other hand, during the more specific interactions taking place between the secondary mesenchyma and epithelia, the tissues are separated by the extracellular environment. Thus, direct contact between unlike cells does not take place.

A rather unique example of epithelial-mesenchymal interactions which takes place between two individuals (the epithelium of the fetal trophoblast and the mesenchyma of maternal predecidua cells) was described by David R. S. Kirby (Oxford University). During the intrauterine implantation of the trophoblast, its invasiveness ceases about the 9th day, when maternal blood vessels are reached and breached. However, the behavior of the trophoblast changes drastically during experimental extrauterine implantation into organs such as the kidney, spleen, and testes; it becomes extremely destructive and the period of invasion is extended. The aggressiveness of the trophoblast seems to be controlled by the formation of decidual tissue, which normally takes place during intrauterine implantation but does not occur in certain pathologic states such as placenta accreta and extrauterine implantation. The degree of trophoblast invasion seems to be governed by the rate of decidual necrosis and is interrupted when the trophoblast comes in contact with a layer of decidual tissue which remains intact throughout pregnancy. Another example of trophoblast-decidua interaction involves the production of luteotrophic hormone. Neither isolated trophoblast nor decidua is capable of producing this hormone. However, luteotrophic hormone is synthesized when trophoblast and decidua are experimentally recombined. This study suggests that during normal pregnancy, the trophoblast exerts an inductive effect upon the secretion of this hormone by the maternal component of the placenta.

A study presented by John W. Saunders (University of Pennsylvania) suggests that a mutual participation of ectoderm and mesoderm is necessary in the differentiation of limb symmetry. He showed that in a limb bud, the mesoderm of the postaxial zone induces the postaxial ectoderm of the apex to form a limb. On the other hand, preaxial mesoderm does not have that inductive effect on the preaxial ectoderm. However, if preaxial ectoderm from the apex is grafted to the postaxial zone, a limb will be formed. Moreover, if the apex of a wing bud is rotated 180° about its proximodistal axis (placing the preaxial ectoderm in contact with the postaxial zone and vice versa) then two wings will be induced. Further, Saunders showed that the mesoderm of the posterior necrotic zone, localized on the posterior edge of the wing bud, when grafted subjacent to the apical ectodermal ridge at the apex of the wing bud, will induce two more-or-less complete sets of wing parts. One set emanates from the pre-

axial portion of the bud, anterior to the graft; and the other set emanates from the postaxial zone, posterior to it. Other areas which undergo necrosis do not bring about this duplication. Moreover, the posterior necrotic zone of the wing or leg bud seems to play a key role in determining the anterior-posterior polarization of the limb parts. However, preliminary studies suggested that the control of the dorsoventral axis may reside in the ectoderm.

Several papers were concerned with the basic mechanisms of cell interactions as they pertain to cellular differentiation and morphogenesis. Howard Holtzer (University of Pennsylvania) suggested that induction is not an event whereby informational molecules are merely transmitted from one cell to another, carrying with them the necessary instruction to convert an undifferentiated cell into a specialized one. Studying the differentiation of somite cells into chondrocytes, he suggested that this process probably involves a sequence of permissive events, whereby only covertly differentiated cells are capable of interpreting the message carried by an inducer. Moreover, he also noted that the events associated with expression of a phenotype are preceded by mitotic activity. This statement was further elucidated by Norman K. Wessells (Stanford University) in his work on the differentiation of pancreatic cells. It is known that to achieve differentiation in vitro of pancreatic cells, embryo extract or mesoderm (homo- or heterotypic) are necessary, suggesting a close but not specific interdependency with extrinsic factors. Since growth is necessary for cells to differentiate in culture, one does not know whether the mesoderm or embryo extract participates actively in cytodifferentiation or act by their ability to support mitosis. Mitosis obviously plays an essential role in growth. However, it was also suggested that some of the division cycles may be a prerequisite for differentiation to take place. Wessells cites two examples in adult systems: (i) immunologically competent cells, after being challenged by an antigen, will divide before production of antibodies takes place; and (ii) cell division of mammary-gland tissues seems to be a prerequisite to the production of caseine following hormone stimulation. He studied the relation of mitosis and differentiation in pancreatic cells by using a DNA inhibitor, 5-fluorodeoxyuridine (FUDR). The effect of FUDR on RNA metabolism may be counteracted with uridine; and its inhibitory effect can be reversed by the addition of thymidine. These experiments showed that when FUDR is added to a culture of dividing pancreatic cells, differentiation will be halted. On the other hand, if FUDR is added to a population of cells where division has already taken place, differentiation will occur despite the fact that tissue growth has been interrupted. These results suggest, without proving it, that terminal divisions may be required for differentiation to occur.

Studies on early interactions taking place during chondrogenesis were presented by James W. Lash (University of Pennsylvania). It is known that somites from explanted embryonic chicks will undergo cartilage formation when exposed to a variety of stimuli such as spinal cord or notochord; mature cartilage undergoing hypertrophy; and extracts of notochord and spinal cord. Under suitable nutritive conditions, somites will form cartilage without additional stimulation although the process is delayed and the amount produced is less than if they were exposed to the stimuli mentioned previously. However, in a 3-day-old embryo, only the spinal cord or notochord can induce somites to produce cartilage. Lash studied the metabolic activities of explanted somites and their response after interaction with a cartilage-promoting agent. He used as a parameter the synthesis of chondromucoprotein. With respect to glucosamine metabolism, somites have the same array of enzymes as mature cartilage; the major difference is that in mature cartilage, glucosamine C^{14} is incorporated into UDP-NA-hexosamines while in somites most of the label is found in glucosamine-6-phosphate and acetylglucosamine phosphates, which are precursors of UDP-acetylhexosamines. It was also noted that sulfation of acid mucopolysaccharides does not take place if somites are cultured alone, unless notochord is added to the culture. Another interesting observation was that in 3-day-old embryos, various tissues besides cartilage are able to synthesize chondroitin sulfate. However, in older embryos (10 days) this synthesis is only detected in cartilage, thus suggesting that synthesis of chondroitin sulfate becomes restricted during development. The studies of Lash suggest that when somites are exposed to various inducers in cultures, no new metabolic pattern is elicited, but a preexisting pattern seems to be enhanced.

William J. Rutter (University of Washington) studied the sequence of events during pancreatic development. He used as molecular markers of differentiation the production of specific proteins synthesized by endocrine and exocrine cells. The production of specific pancreatic proteins was correlated with the development of morphogenetic characteristics. On the basis of this investigation, it was possible to demonstrate the following stages: (i) an undifferentiated stage where synthesis of pancreatic specific proteins is essentially zero; (ii) a primary regulatory event whereby an undifferentiated cell is converted into a cell with pancreatic potential, this in part mediated through the pancreatic mesenchyma; (iii) a protodifferentiated state where production of specific pancreatic proteins of endocrine and exocrine origin becomes detectable (this is accompanied by early expression of morphogenesis); (iv) a secondary regulatory event which involves the conversion of protodifferentiated cells to a differentiated stage; cell proliferation ceases in those cells undergoing differentiation and synthesis of the endoplasmic reticulum begins; (v) the achievement by the cells of a full state of differentiation; and (vi) a tertiary regulatory event, whereby differentiated cells are subjected to modulation of enzyme concentrations by external stimuli.

Charles E. Wilde (University of Pennsylvania) presented data suggesting that morphogenesis is the result of the transcription and translation of morphogenetic information encoded in messenger RNA. Morphogenetically meaningful RNA synthesis takes place immediately after fertilization although the messages are retained for 40 hours or more and expressed with the onset of gastrulation. In lower vertebrates, transcription of morphogenetic messages depends upon the supply of ATP, by means of aerobic metabolism; glycolytic energy is not capable of supporting this function. Thus, ATP synthesized by glycolysis, although present in sufficient levels, does not support the synthesis of RNA during cleavage stages. Wilde's data also suggest that selective protein synthesis occurs during cleavage stages even if aerobic metabolism and RNA synthesis are completely arrested.

A. A. Moscona (University of Chicago) discussed the problem of cell

communications as they relate to the patterns of associations and organization of cells into well defined structures. Dissociated skin cells of 8-day-old embryonic chicks, when placed in the chorioallantoic membrane, will form feathers. Older cells (12 to 14 days) will only form keratinized structures but no feathers. If skin cells are mixed with a heterologous population (liver, lung, kidney, or heart cells), the formation of feathers is completely suppressed, thus suggesting an incompatibility between different phenotypes. Moscona also studied interactions between cells of different genotypes, namely, skin cells of chicks and mice. When a mixture of skin cells (capable of forming hair follicles) from a 13-day-old mouse and embryos (capable of forming feathers) of 8-day-old chicks was placed in the chorioallantoic membrane, the following structures were produced: (i) feathers and hair follicles, (ii) sheets of cysts from either chick or mouse cells or chimeric mosaics with epidermal cells from both species, and (iii) feathers with mouse epidermal cells. On the other hand, chick epidermal cells never participated in hair follicle formation. When mouse epidermal cells (dermis removed by trypsinization) were mixed with total chick skin cells, their behavior was similar to that described previously. In addition, there were downgrowths of mouse epidermis attempting to form hair follicles which were associated with condensations of chick dermal cells, thus suggesting that induction was taking place with these genotypically different cells. When chick skin cells were mixed with epidermal or dermal mouse cells 13 days old, there was no interference with feather formation. However, if the mouse cells were older than 14 days, feather formation by the chick skin cells was suppressed. The author concluded that 14-day-old mouse skin cells have already established their phenotypic specificity so that they cannot participate in functions programmed in a different genotype.

The formation of interface materials during epithelial-mesenchymal interactions and their possible role in morphogenesis was discussed by Clifford Grobstein (University of California). When epithelia interact with mesenchyme through a Millipore filter, collagen fibers accumulate at the surface of the epithelium. Removal of the collagen fibers by collagenase seemed to interfere with epithelial morphogen-

esis. Autoradiographic data showed that the collagen was being synthesized by the mesenchyma. Further investigations on uptake of tritiated glucosamine showed that the epithelium was the site for synthesis of a mucopolysaccharide, susceptible to hyaluronidase digestion. The mucopolysaccharide probably participates in the process of fibrinogenesis at the surface of the epithelium. Thus, the data imply a two-way interaction between epithelia and mesenchyma in the formation of interface materials. Robert S. Hilfer (Temple University) described the interactions taking place between secretory and capsule cells in the thyroid of embryonic chicks. When 8-day-old epithelial thyroid cells are dissociated, spread in monolayer culture, and subsequently reaggregated, they fail to develop a canaliculate endoplasmic reticulum. Further, similarly treated older cells (18-day-old) lose their already organized endoplastic reticulum. However, if the reaggregation of spread thyroid epithelial cells (8 or 18 days old) are mixed with thyroid capsule cells, the epithelial cells develop into a normal thyroid pattern. This epithelial-mesenchymal interdependency seems to be specific since fibroblasts from the thyroid capsule cannot be substituted for fibroblasts from mesentery, heart, or perichondrium. This inductive effect of the thyroid mesenchyma also takes place through a Millipore filter.

Robert Auerbach (University of Wisconsin) discussed the interactions between the thymus gland, spleen, and bone marrow as they relate to lymphoid cell differentiation. Following sublethal irradiation of the spleen, its immunological reactivity can be restored by the thymus gland even if both tissues are separated by a Millipore filter. However, if a lethal dose of irradiation is given to the spleen, its immunological competence can only be restituted under both thymic and bone marrow inductive influences. It was also shown that when bone marrow is grown in vitro, lymphoid cells can only survive and proliferate if thymus tissue is added to the culture. This influence is also exerted through a Millipore filter. The same interdependency exists in the opposite direction; bone marrow influences lymphoid differentiation of the thymus gland. Moreover, the spleen may also exert a stimulatory effect on the lymphoid tissues of the thymus and bone marrow. The AKR mouse strain routinely develops lymphocytic leukemia at age 6 to 12 months. Prelim-

inary studies indicated that the thymus from the AKR mouse is more effective in stimulating lymphopoiesis in bone marrow than the thymus from nonleukemic lines (C_3H). Since mesenchymal influences are responsible for thymus lymphoid differentiation, Auerbach raises the question as to whether leukemogenic changes may have occurred through an inductive affect of altered mesenchymal cells.

Sister Muriel Lippman (Nazareth College) is primarily concerned with the effect of natural acidic glycosaminoglycans in cell division. In her earlier work, she showed that heparin reduces mitotic index and tumor growth in Ehrlich ascites carcinoma. Several acid mucopolysaccharides were tested for their ability to reduce growth of mouse L-cells in suspension cultures. All of them including hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparitin sulfate, keratan sulfate and heparin acted as inhibitors of growth in varying degrees. Since most acid mucopolysaccharides in vivo are bound to a protein, a protein-polysaccharide complex (PP-L) obtained from bovine nasal cartilage was tested for its inhibitory effect on growth. The PP-L complex contains about 90 percent chondroitin-4-sulfate and about 10 percent of keratan sulfate. Both polysaccharides have marked inhibitory effects on growth. The results of this experiment were rather intriguing since the PP-L complex showed an initial marked stimulatory effect on growth rate, followed later by an inhibitory effect. Such results suggest that the protein fraction of the complex was already metabolized and let the free polysaccharide exert its inhibitory effect. Sister Lippman also showed that the polyanion polysaccharides are bound to the cell surface. In this regard, Ehrlich ascites cells treated with hyaluronic acid or heparin, and untreated controls, were injected into allogeneic or syngeneic hosts. While the untreated cells were promptly rejected, the treated ones developed into enormous tumors which metastasized and were transplantable. This suggests that the treated cells coated by the test material were not recognized as foreign by the host and consequently not rejected.

Ruppert E. Billingham (University of Pennsylvania) discussed the preservation of epithelial specificity through mesenchymal influences. A series of heterotypic recombination grafts from guinea pig skin (that is, epidermis from

the ear combined with dermis from the sole) were transplanted to an appropriate host. Histologic examination of these combined grafts strongly suggested that epidermal specificity was determined by the underlying dermis. On the other hand, epithelia from mucosae (tongue or esophagus) retained its original characteristics when recombined with ear or sole dermis. However, when mucosal epithelium was recombined with trunk dermis, it acquired the characteristics of trunk epidermis. In order to study cytodifferentiation and morphogenetic potentials of epidermal cells in a nondermal mesenchymal environment, suspensions of epidermal cells were inoculated in muscle, spleen, and beneath the renal capsule. Histologic examination of these cellular implants revealed not only formation of epidermal cysts, but more complex structures. Sebaceous glands and hair follicles with papillae, surrounded by a connective tissue with a structure resembling that seen in the dermis, were noted.

The behavior of adult epidermal cells in vitro and in vivo, as it relates to organization, differentiation, and mitotic activity was reported by Eugene J. Van Scott (National Institutes of Health). Adult epidermal cells cultured in a suitable medium and placed in contact with a glass or plastic surface develop, after 2 to 3 weeks, an outgrowth of several layers with a distinct gradient of cell maturation. Mitotic activity was seen only in the first two lower layers of basal cells. The next three to four layers consisted of basal cells, whereas the uppermost layers, in contact with the nutrient medium, consisted of mature epidermal cells undergoing keratinization. Thus, adult epidermal cells in vitro can organize and differentiate (tonofibrils present) in the absence of connective tissue. However, in these experiments, keratohyaline granules and a stratum corneum did not develop. This study suggests that the connective tissue may play a role in promoting the full manifestation of epidermal cell behavior. The control of mitotic activity of the germinative cells in the hair follicles is determined by the surface area of the dermal papilla, since only those cells in contact with it divide. Keratinization or cell death takes place when a follicular cell is separated from the stroma by a distance of 100 microns. Further studies on the interdependency of the follicular epidermis and its corresponding papilla were re-

ported by Roy F. Oliver (University of Birmingham, England). He showed that implants of follicular epidermis from vibrissae, where the tubular arrangement was preserved, would regenerate a papilla and whisker while similar implants of flat follicular epidermis failed to do so. Thus, the spacial arrangement of follicular epidermal cells seemed to be a prerequisite for morphogenesis to take place. Transplantation of vibrissa dermal papillae to the upper half of vibrissae follicles induced whisker growth. However, induction of follicle or hair formation did not take place when epidermis from the ear (which contains hair follicles) or from afollicular scrotal sac epidermis were implanted into ear skin in proximity with vibrissa dermal papillae. Both types of epidermis did, however, become organized locally into "matrices" around the papillae. This lack of inductive effect may be due to several factors. The stimulating effect of the papillae was not intense enough; some epithelia are more refractory to dermal influences than others; and the effect of local dermal influence(s) at the site of implantation overrides the inductive properties of the vibrissa dermal papillae.

Clyde J. Dawe (National Institutes of Health) reported that the induction, in vitro, of tumors in salivary gland rudiments by polyoma virus, requires the presence of both epithelium and mesenchyma. If trypsin-isolated epithelial and mesenchymal components are exposed separately to the polyoma virus, neither component causes the development of tumors. The appearance of tumors in the salivary gland rudiments was accompanied by some morphogenetic changes of the epithelium. These experiments also revealed that tissue from polyoma-virus induced tumors is capable of supporting growth and normal adenomere formation of isolated salivary gland epithelium. It is not known whether this morphogenetic effect is due to the neoplastic or to the stromal components of the tumor.

Johannes Holtfreter (University of Rochester) and C. B. McLoughlyn (University College, London) were unable to attend the meeting but their contributions will be included in the publication of the full-length papers. The Williams and Wilkins Company will publish the proceedings.

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