# Direct Metabolic Interactions between Animal Cells

## Their role in tissue function and development is reconsidered.

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Multitudinous forms of cellular interaction are among the basic prerequisites for the continuity of life. The life cycle of unicellular organisms, like bacteria, depends upon a wide range of saprophytic and epiphytic relations, and sexual reproduction in protoza is initiated by mating reactions which are determined by specific molecular patterns in the surfaces of interacting cells (*1*). Some organisms, like slime molds, exist during part of their life cycle in a unicellular state, in another part of their life cycle in a multicellular state. The transition from the former to the latter state involves a definite pattern of cell interactions which results in cell aggregation and the subsequent differentiation of the cells in the aggregate (*2*).

The evolutionary appearance of strictly multicellular organisms can be considered an elaboration and consolidation of protistan forms of cell interactions. An obvious aspect of cell interactions in multicellular organisms is the continued contact between cell surfaces (*3*). This phase of cell interaction is being investigated by electron-microscopic analysis of cell boundaries and of basal cell membranes (*4*), by the study of adhesion (*5*) and of aggregation and disaggregation of cells (*6*), and by the study of the behavior of cells in tissue culture (*7*). A molecular basis for the interaction of cell surfaces has been considered (*8*).

Particular importance has been attributed to the role of direct cell interactions in the process of embryonic development. Actually, the bulk of the work carried out in experimental embryology consists of attempts to demonstrate the appearance of morphological changes in one cell type in response to the presence of another cell type under a variety of in vitro and in vivo conditions. In recent years much ingenuity has been applied in work with systems of embryonic tissues in which characteristic forms of cell interaction can be demonstrated experimentally (*9*, *10*). Much can be gained in this work with embryonic tissues, since the cell interactions which occur during embryonic development constitute one of the ways in which the genetic inventory of the primitive embryonic cell comes to expression and determines the appearance of certain cell properties which are characteristic for the fully developed cell.

Although some advance has been made in understanding the nature of the changes that occur during and subsequent to cell interaction, little consideration has been given to the relation of the observed structural changes to alterations in the metabolic pathways during cell interactions. One exception is perhaps the analysis of fertilization in the sea urchin. How much of the information obtained in this instance is applicable to cell interactions in general is uncertain.

It is proposed in this article to discuss briefly two types of drastic metabolic readjustments which are part of the interaction of animal cells and to consider some of the implications of these findings. Interaction is essential in one case for the functional activity of the interacting cells; in the other case, for maintenance of metabolic synthesis.

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### Metabolic Interaction and Specific Function

The bulk of the evidence for functionally significant metabolic interactions between cells is derived from work, carried out about 20 years ago, on the production of fluid in the brain by the chorioplexus (*11*) and the formation of aqueous humor in the eye by the ciliary body (*12*) These histologically heavily folded tissues consist of a single and double layer, respectively, of closely packed cuboidal epithelial cells which cover a loose mesodermal connective tissue, the stroma, in which the cells are separated from each other and from the epithelial layer by collagen fibers and a ground substance (Fig. 1). In surviving segments of these tissues it was found that the epithelia showed strongly positive oxidation-reduction potentials and the stromata, strongly negative oxidation-reduction potentials when tested with a large series of oxidation-reduction indicators with closely spaced potentials. Under anaerobiosis, or in the presence of sodium cyanide, the epithelium acquired a strongly negative oxidation-reduction potential. This indicated the dependence of the positive values for oxidation-reduction potentials on oxidative enzyme systems in this tissue component. The stroma was assumed to be the site of dehydrogenases catalyzing reactions with strongly negative potential and to be devoid of oxidative enzymes such as cytochrome oxidase. A mechanical separation of the epithelial and stromal cells in these tissues and manometric determinations of enzyme activities in the two tissue components confirmed this interpretation of the results obtained with the oxidation-reduction indicators (*13*).

The functional significance of the differences in oxidation-reduction potential became apparent from two lines of evidence. In the embryonic chorioplexus, no potential difference was found between epithelium and stroma before the onset of functional activity (*14*). The potential difference and the production of cerebrospinal fluid became established at the same stage of fetal life. Also, in the fetal kidney analogous changes in the cytochrome-oxidase activity in the tissue components were observed with the beginning of functional activity (*15*). In another series of experiments a shift in the potential differences of the ciliary body

was observed in scorbutic guinea pigs (*16*).

The appearance of differences in oxidation-reduction potential during embryonic development was brought about by an increasing concentration of oxidative enzyme systems in the epithelia of these tissues and the maintenance of negligibly low concentrations of oxidases in the stromata. The shift in the potentials in the scorbutic animals was explained by a break in the metabolic interaction of the epithelium and stroma. It was proposed that ascorbic acid is an essential mediator in the electron transport from the epithelium to the stroma and that its absence in a deficient organism leads to an interruption of the electron flow from one tissue component to the other.

From these experiments it was concluded that the undisturbed maintenance of the potential differences in the epithelium and stroma of the ciliary body, of the chorioplexus, and also of the kidney is essential for the functional activity of the tissue. The metabolic maintenance of these potential differences generates a flow of electrons from the stroma to the epithelium and becomes a source of energy for transport of fluid or of ions between these tissues. In this sense the maintenance of the potential difference is essential for the continuation of the function of these tissues.

It should be pointed out that more than metabolic oxidation-reduction reactions can be involved in tissue interactions. Two adjacent tissue components may also participate in functionally important metabolic transformations of specific molecular cell constituents. Such a case is the cycle of visual pigment in the retina, in which both the neural epithelium and the pigment epithelium participate (*17*).

## Dependence of Mesodermal Syntheses on Epithelial Metabolism in the Cornea

In the cornea, a more systematic quantitative study of intercellular metabolic reactions is made possible by the ease with which stroma and epithelium can be mechanically separated. This is due to the smoothness of the boundary between the two tissue components over a large part of the corneal area (Fig. 1). As in the tissues mentioned above, the enzyme activities involved in the terminal metabolic oxidations were found in the epithelium. High rates of glucose utilization and lactic acid formation were found in both the epithelium and the stroma (*18*). In following the changes in the lactic acid level, it was observed that the lactic acid in the stroma decreased much more slowly in the absence of epithelium then it did when epithelium was present. It was concluded that a part of the lactate which is formed in the stroma is oxidized in the epithelium. Thus, it seemed that the stroma supplied a substrate for enzymatic oxidations in the epithelium.

For a long time the question of whether the stroma benefits in any way from supplying raw materials for oxidative processes in the epithelium remained unexplored. Within the last two years, however, evidence has accumulated that two, and perhaps three, synthetic processes in the corneal mesoderm are greatly reduced if the epithelium is removed from its close juxtaposition to the stroma. The following syntheses in the stroma were found to be affected by the removal of the epithelium: (i) the incorporation of glycine-1-$C^{14}$ into the collagen fraction (*19*); (ii) The incorporation of $S^{35}$ into the mucopolysaccharides of the ground substance (*20*); and tentatively, (iii) the uptake of thymidine-$C^{14}$ into the hot trichloroacetic acid extracts (deoxyribonucleic acid) of the stroma (*21*). This apparently means that metabolic processes in the epithelium can substitute for incomplete metabolic pathways in the stroma and that in this manner the epithelium can maintain synthesis of macromolecules in the stroma.

## Mechanism of the Epithelial Effect

Investigations carried out so far provide only tenuous clues as to the nature of the interaction between the epithelium and the stroma. Addition of a homogenate or a mince of the corneal epithelium, of other chick organs such as liver, heart, or kidney, or of adenosine triphosphate to the incubation mixture used in incorporation measurements was found to be ineffective as a source of energy for restoration of synthetic activity in the stroma after removal of the epithelium (*22*).

Such experiments make it doubtful that diffusible metabolic intermediaries such as high-energy phosphate esters or coenzymes serve as links in the transmission of metabolic energy from the one to the other tissue component. These doubts are strengthened by autoradiography of corneal sections incubated with $C^{14}$-labeled glycine solution after removal of one half of the epithelial cover of the stroma (*23*). Such preparations show that incorporation into the stroma follows a sharp boundary which coincides exactly with the termination of the epithelial cover of the stroma. The abruptness of this boundary is not compatible with an assumed diffusion gradient. Therefore, one can ask whether the transmission of metabolic energy from the epithelium to the stroma follows some structural component of the stroma—for example, protein-sulfhydryl groups—or whether it involves small molecular compounds (perhaps ascorbic acid) which are bound to some structural elements of the mesodermal matrix. In this connection it should be pointed out that several systems involving protein-sulfhydryl groups and bound water for electron transport over a distance have been suggested recently (*24*). The role of sulfhydral groups as terminal acceptors for electron transfer from mitochondria to the bacterial cell wall has been investigated by Falcone and Nickerson (*25*).

Attempts were made to distinguish, by the use of metabolic inhibitors, between metabolic pathways supporting syntheses in the epithelium and in the stroma, respectively. Although these experiments have not led to conclusive results as yet, differences between the effects of some metabolic inhibitors on protein synthesis in the stroma and in the epithelium seem to indicate differences in these pathways (*26*). At the same time, analysis of changes in the metabolic systems in the stroma in the absence of the epithelium are being carried out in order to obtain more direct information about those metabolic reactions in the stroma which are dependent upon the epithelium and about the pathways of energy transfer from the epithelium to the stroma.

The cornea may be a model system for the exploration of mechanisms of certain metabolic cell interactions since it seems to be the first tissue in which a dependence of protein synthesis on tissue interaction can be observed. The main advantages of this system for a far-reaching analysis of such metabolic interactions are its accessibility *in situ*; the ease of preparation of the two tissue components, which can be used to obtain quantitative information about the effect of the epithelium on the stroma;

the viability of the cornea *in vitro*, the homogeneity of the cell populations in the separable tissue components; and the specificity of the cell products synthesized in the stroma (collagen, keratosulfate).

### Role of Metabolic Tissue Interactions in Developmental Processes

The importance of the metabolic tissue interactions which are necessary for the function of these tissues is evident. It remains to define the possible significance of the metabolic tissue interactions which maintain synthetic processes in one or in both of the involved tissue components. The studies on the cornea call attention to the role which metabolic tissue interactions may play in the course of embryonic development.

The dependence of the corneal stroma upon the epithelium has been observed on the eighth day of development of the chick embryo (*27*). This is only two days after the onset of cell migration from the head mesenchyme into the corneal area. Therefore, it is evident that protein synthesis and, consequently, growth of the corneal stroma early become dependent on the supply of epithelial metabolic energy. In contrast, the stationary cells of the head mesenchyme, which form the sclera, develop their own enzymatic mechanisms for production of the energy needed for synthetic processes. As a consequence, the scleral mesoderm can grow autonomously.

Even before differentiation of the corneal and scleral mesoderm takes place, the head mesenchyme must produce enough energy to support the syntheses required for proliferative growth. The apparent loss of this autonomy in the mesenchymal cells which become corneal mesoderm can come about through one or both of the following changes.

1) The total energy output of a primitive mesenchyme cell may be sufficient to support the synthetic activities connected with the growth and differentiation of these undifferentiated cells into corneal mesoderm. In this case, the necessity for a supply of energy from the epithelium for synthesis would mean that some of the energy produced in these differentiating cells is diverted into functional channels such as movement of fluid. A scheme for this type of distribution of energy consumption for functional or growth processes has recently been discussed by Rutter (*28*).

2) The need for an epithelial energy supply to the stroma could also mean that the enzyme systems generating energy deteriorate to some extent as the head mesenchyme differentiates into the corneal mesoderm. Such a loss is apparent from the results of Kuhlman and Resnik (*29*). These authors found that in new-born rats the isocitric dehydrogenase and glucose-6-phosphate dehydrogenase activities in the corneal epithelium increased sharply, while the corresponding enzymatic activities in the stroma declined with development. Other examples of protein loss during differentiation are the disappearance of heart myosin from parts of the blastoderm (*30*) and of proteins for the formation of tryptophan peroxidase in the embryonic gut (*31*).

The scleral and corneal mesoderm differentiate not only with respect to autonomy or dependence of their energy supply but also with respect to the type of mucopolysaccharide produced. In the nontransparent sclera of the chick, cartilage is formed with chondroitin sulfate as the main constituent. In the cornea is found a mucopolysaccharide mixture in which keratosulfate is prevalent. Chondroitin sulfate contains galactosamine, glucuronic acid, and sulfate, while keratosulfate is made up of glucosamine, galactose, and sulfate but no uronic acid. It will be of interest to investigate whether the same differences in the metabolic pathways which support syntheses in the cornea and sclera, respectively, also control the types of mucopolysaccharides which are formed

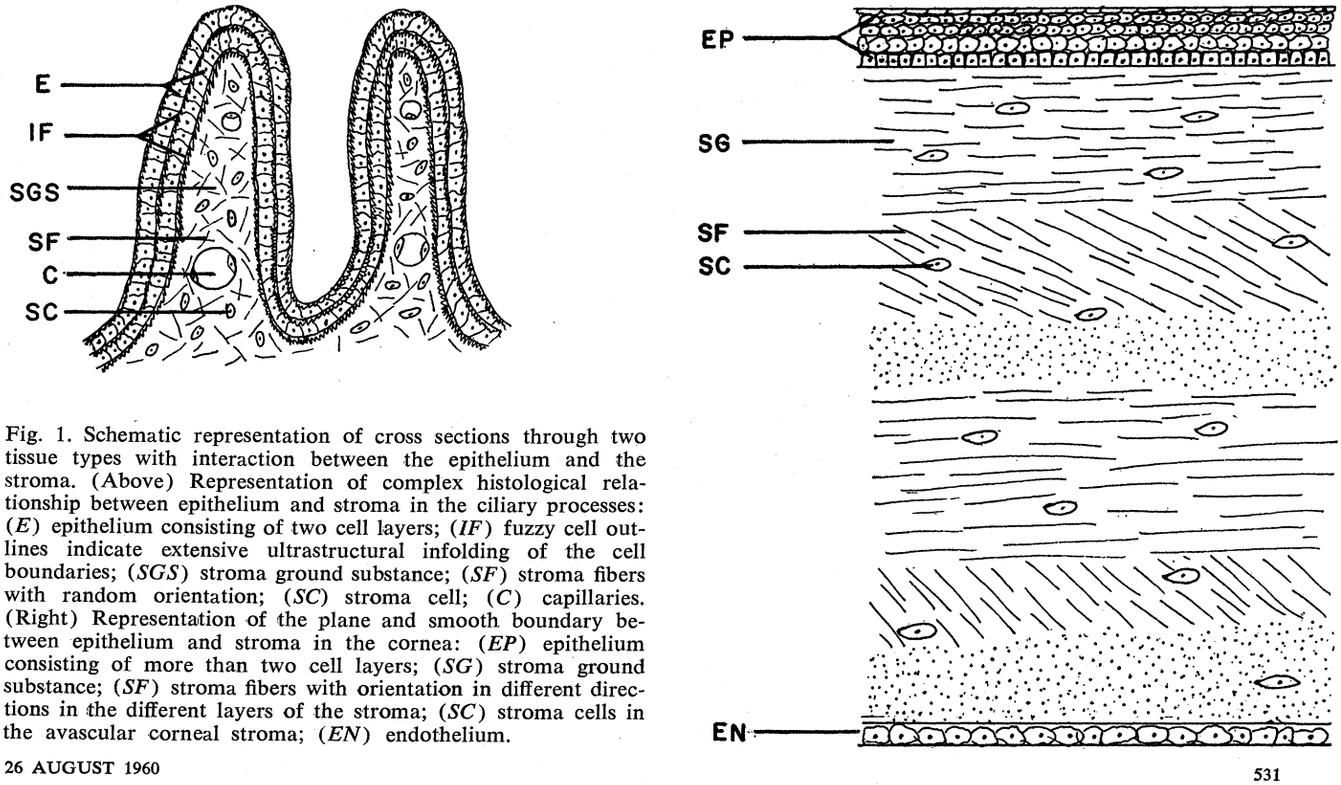


Fig. 1. Schematic representation of cross sections through two tissue types with interaction between the epithelium and the stroma. (Above) Representation of complex histological relationship between epithelium and stroma in the ciliary processes: (*E*) epithelium consisting of two cell layers; (*IF*) fuzzy cell outlines indicate extensive ultrastructural infolding of the cell boundaries; (*SGS*) stroma ground substance; (*SF*) stroma fibers with random orientation; (*SC*) stroma cell; (*C*) capillaries. (Right) Representation of the plane and smooth boundary between epithelium and stroma in the cornea: (*EP*) epithelium consisting of more than two cell layers; (*SG*) stroma ground substance; (*SF*) stroma fibers with orientation in different directions in the different layers of the stroma; (*SC*) stroma cells in the avascular corneal stroma; (*EN*) endothelium.

in these two tissues and determine thereby their histological character.

The results obtained through analysis of the corneal tissue interaction should be directly pertinent to an understanding of embryonic systems in which growth and differentiation of a mesodermal component depend upon the presence of ectodermal cells. This could be expected to be true particularly in the developing limb (*10*) and wing bud and in some forms of limb regeneration (*32*). In this connection, several investigators have found that the normal development of the mesodermal skeleton depends upon the presence of a specific thickening in the ectodermal cover of these primordia. In turn, in the absence of an appropriate mesodermal component, thickening of the ectodermal cover does not occur. Other types of embryonic ectodermal-mesodermal interactions, such as those between somites and neural tissue (*33*) and those observed in several in vitro systems (*9*), may show more or less closely related mechanisms as part of the total differentiation process.

The demonstration of pathways of metabolic interactions between cells may lead to a reconsideration of earlier thoughts about the role of metabolism in morphogenesis, such as the gradient theories of Runnstrom (*34*) and Child (*35*). The significance of these theories was never fully appreciated because they failed to establish a relation between the observed metabolic parameters and synthetic processes which determined, at least in part, the progress of development in the analyzed tissues. With the recognition of a control of syntheses by intercellular metabolic interactions, these early observations may become more meaningful. Also, new and pertinent data have been obtained by Spratt (*36*) and by Backstrom (*37*). It is of particular interest that many data in this work point to sulfhydryl groups of small molecular substances and of proteins as the key to the metabolic control of embryogenesis. In view of work on sulfhydryl proteins mentioned above (*24*), it may be that large molecular sulfhydryl compounds constitute a component in the unification of structural and functional aspects of developing tissues. Possibilities of a control of protein synthesis in one tissue by supply of essential amino acids from an adjacent tissue have been pointed out by Wilde (*38*).

Metabolic cell interactions may also occur in adult tissues, although the slow rate of syntheses may make their detection more difficult. Nevertheless, a more intensive search for such possibilities in adult tissue seems desirable, since some degenerative processes may well be brought about by a failure of just such direct metabolic interactions between cells (*39*).

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