Recent Studies on Corneal Metabolism and Growth: A Review

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There is hardly a treatise on oncology that does not begin with the definition of cancer as a tissue or group of cells that has in some degree escaped from the "normal" restraints of tissue growth and is growing "autonomously." One searches in vain, however, for any clear insight into the nature of these normal restraints and their mode of operation. The problem is touched upon tangentially in the field of hormone research and is central to developmental embryology where, unfortunately, the issue has been clouded by the somewhat mythical concept of organizers. No disparagement is meant here of the truly monumental work of the past generation in the field of experimental embryology, for in the embryo everything is growing and differentiating and changing simultaneously, so that there is no fixed point of reference from which the factors governing the changes in a particular group of cells can be evaluated. It was most natural to assume that these multitudinous effects might be mediated by some specific organizers, and this possibility has not been definitely excluded even by a generation of unsuccessful attempts to isolate and characterize such organizers.

More recent trends of thought indicate a growing awareness of the multiplicity of possible metabolic interactions of adjacent cells and tissues, and a theoretic basis for differentiation resulting from such interactions has been provided by Spiegelman (42). Drawing implications from his work on yeast, Spiegelman has suggested that cells acquire their characteristic pattern of enzymes by virtue not only of their genetic potentialities but also, specifically, under the influence of available substrates or metabolites; that due to substrate gradients in the embryo, cells in different locations acquire different enzyme content; and that in this differentiation they further alter each other's environment so as to lead to still further differentiation. It would seem that the test of Spiegelman's hypothesis would be most difficult in the field of embryonic development, where everything is changing at once, and might be pursued more fruitfully in relation to adult tissues, where everything except those factors under specific observation can be kept in relatively stable homeostatic balance.

It is in the perspective of these broad questions that some of the recent work on the cornea is to be reviewed. This tissue is perhaps one of the simplest organized structures in the mammalian body, but even the preliminary and halting steps that have so far been taken in its study reveal a high degree of interaction of its neighboring components. At this early stage no very general and well defined patterns have as yet become visible, but even these small beginnings may serve to stimulate similar studies on other tissues and to make clear how long is the road ahead toward an understanding of the normal restraints of tissue growth which lead to orderly and organized structure, and from which cancer cells somehow escape into "autonomous" growth.

Metabolic organization.—The main bulk of the cornea is the collagenous stroma sparsely supplied with cells. This is covered on its anterior surface by a layer of epithelium four to six cells deep, and on its posterior surface by a single layer of mesothelium. Since the epithelium or mesothelium can be scraped off with little damage to the stroma, it is possible to compare the metabolism of the separated layers with that of the intact tissue.

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Herrmann and his co-workers (27-30) found that the epithelium contains glycogen, has a cyanidesensitive respiration, and a conventional glycolytic and glucoytic mechanism. The corneal stroma, on the other hand, has no oxygen uptake but consumes as much glucose per cell as does the epithelium. The product of this glucose consumption is lactic acid. In the intact cornea the lactic acid produced by the stroma is consumed by the epithelium and constitutes one-quarter or more of the total carbohydrate utilized by these cells.

The denuded stroma, on incubation, rapidly loses its capacity to glucolyze, the process coming to an end when all the available inorganic phosphate has been incorporated into triose phosphate. It is evident that this tissue lacks a phosphate acceptor, presumably adenosine diphosphate, needed for the conversion of triose phosphate to lactate, and for the continued operation of the glucoytic cycle. Thus, the metabolic relation between the epithelium and stroma is a reciprocal one, in which the epithelium receives lactate and simultaneously delivers to the stroma a necessary co-factor for glucolysis.

There is some evidence suggesting that the transfer of lactate from stroma to epithelium is an active one, not simply passive diffusion. Butyrate and serine injected into the stroma are not utilized by that denuded tissue but in the intact structure are consumed at approximately the same rate as lactate. The utilization of stroma lactate, butyrate, and serine by the epithelium are all inhibited by the same concentration of mustard gas. It would appear that the stroma-epithelium boundary is the locus of highly complex metabolic activity.

Cogan and Kinsey (8, 9) have shown that this boundary has highly specialized permeability characteristics, being permeable to water and to lipoid-soluble substances but relatively impermeable to crystallloids. There is no necessary contradiction between the active transport of certain substances across a boundary and the impermeability of that boundary to diffusion of these same or similar substances. In fact, if active transport is to be effective, it must be associated with an impermeability to diffusion of the transported substance, since otherwise back diffusion would very greatly diminish the net amount of substance transported. The mechanism of active transport in this case has not been investigated.

**Tissue cohesion.**—An important aspect of the interrelration of adjacent tissues is that of cohesion. It would appear that this attribute is not without significance for some aspects of invasiveness of tumors and for the relation of some tumors to their own stroma. This subject has in the past received only scattered attention. The general literature in this field has been summarized by Buschke (4).

The adhesion of the corneal epithelium to the underlying stroma was studied by Herrmann et al. (24-26). Among the substances which cause a loosening of the corneal epithelium are those of detergent type, including compounds of such low molecular weight as butyl alcohol. The authors were led by this finding to the conclusion that the cohesive boundary includes a lipid component. On the other hand, proteolytic enzymes, e.g., trypsin and chymotrypsin, also resulted in loosening of the epithelium, indicating a protein component of the cohesive boundary. Inorganic salts and wide variation in pH had little effect, suggesting that acid-base bonds played no significant role in the cohesive mechanism. Exposure to certain metabolic poisons and subsequent incubation resulted in loosening of the epithelium. Similar results were obtained following exposure to such vesicating agents as freezing and mustard gas. The authors concluded that the maintenance of coherent organization may require metabolic energy, but the possibility that proteolytic enzymes might be activated under these circumstances could not be excluded. Local anesthetics are particularly effective in causing epithelial loosening. Herrmann et al. (31) found that local anesthetics were powerful inhibitors of respiration in the corneal epithelium, but this did not explain their influence on tissue cohesion, since cyanide also suppressed respiration but did not result in looseniing. Exposure to very low concentrations of histamine resulted in a loosening of the superficial layers of the corneal epithelium, leaving the basal layer still firmly adherent to the stroma.

The cohesion of the corneal epithelial cells to one another has been studied in some detail by Buschke (4, 5). Trypsin, chymotrypsin, and papain greatly decreased the intercellular cohesion, while lysozyme, hyaluronidase, ribonuclease, and crotoxinlipase were without effect. The effect of the proteolytic enzymes was antagonized by calcium and, to a lesser degree, by other alkaline earth elements and by lithium. Iodide and thiocyanate tend to diminish this calcium effect. Anionic detergents were found to abolish intercellular cohesion, but cationic and non-ionic detergents were without significant effect. Buschke has studied the influence of various metabolic poisons on the intercellular cohesion. Exposure to fluoride, iodoacetate, iodoacetamide, 2,4-dinitrophenol and 4,6-dinitroresol, and some local anesthetics, followed by subsequent incubation, led to a loss of cohesion. Cyanide, malonate, and azide were in-
effective. Quinone and trinitrophenol were very effective, and exposure to these agents led to dissociation of the corneal epithelial sheet into a mass of isolated cells, after only brief incubation. It is evident that there are some similarities but also some major differences between the forces that bind the epithelial cells to one another and the forces that bind the epithelial sheet to the underlying stroma.

**Mitotic activity.**—Studies on the mitotic activity of the corneal epithelium have been undertaken by a considerable number of investigators. The literature in this field has been recently reviewed by Friedenwald (10) and Buschke (3). Many of these studies were undertaken for the immediate, practical purpose of evaluating local toxic effects of the commonly used medications, antibiotics, etc., and have little direct relevance to the cancer problem. A useful and simple method of mitotic assay in this tissue has been worked out by Buschke, Friedenwald, and Fleischmann (6), and has been widely used by other investigators with generally concurrent results.

Mitotic activity in the corneal epithelium is limited to the two most basally located cell layers. In the rat, the animal of choice for most of this work, the process of mitosis in these cells requires approximately 70 minutes. The intermitotic period is approximately 200 hours. This long interphase renders this tissue of some special value for comparison with those others, mostly characterized by quite short interphases, that have been used in the past for quantitative mitotic studies. There are many inhibitors of mitosis whose effect is predominantly or exclusively that of prolonging interphase. In relation to some of these, the normally long interphase of the corneal epithelium renders this tissue especially useful for the investigation of threshold effects. Agents in respect to which the threshold of mitotic inhibition have been fruitfully investigated are mustard gas and related compounds (15, 20, 21), ultraviolet (7, 17) and x-radiation (19, 41), and adrenalin (13).

Pin-prick wounds of the corneal epithelium cause an inhibition of mitosis in the adjacent uninjured cells (12). Cervical sympathectomy diminishes the mitotic rate and also slows down the mitotic cycle (13). Similar effects have been found in vitamin A deficiency (18) and in barbiturate anesthesia (14). Local anesthetics and sympathicomimetic drugs are powerful inhibitors of mitosis (14). Several vitamin deficiencies (ascorbic acid, riboflavin) have been found to be without effect on corneal mitotic activity even when the growth of the animal as a whole has been severely stunted (14). The effect of age has been studied by Smelser (37), but, outside of this, the large and interesting field of endocrine effects has not been explored.

Buschke, Friedenwald, and Moses (7) found that threshold exposures to a low-pressure, quartz mercury vapor arc caused an increase in mitotic activity in the rat's corneal epithelium. Further analysis of this phenomenon (17) revealed that the effective stimulating agent was not the radiation emitted by the arc but some gaseous component, possibly ozone, in the atmosphere surrounding the arc. No stimulating effect was found in an extended investigation of the whole accessible ultraviolet spectrum when the possibility of ozone contamination was excluded. The possible bearing of these findings on the much disputed “mitogenetic” rays of Gurwich deserves consideration.

Agents which stimulate or retard mitotic activity are of interest in that they may furnish clues to the normal restraints on growth by which the orderly relation of the tissues is maintained. Unfortunately, those agents thus far studied have not been particularly illuminating in this respect.

Of more immediate and practical interest to the oncologist are those mitotic poisons that cause death of rapidly growing tissues. The mode of cellular death produced by these agents—nuclear fragmentation or karyorrhexis—has been studied in many organs and tissues. It is of some interest that three of the most extensively studied agents of this type, ionizing radiation, ultraviolet radiation, and the nitrogen mustards (19, 21), show wide differences in this regard on the rat's corneal epithelium. Ionizing radiation, although capable of producing karyorrhexis in many other tissues, fails to produce this effect in the corneal epithelium even when administered in doses up to 8,000 r. The nitrogen mustards produce karyorrhexis in a small number of cells in the basal epithelium. Friedenwald, Buschke, and Scholz (21) have advanced evidence, admittedly indirect, suggesting that the cells so affected are those that were in premitosis at the time of exposure. The dosage of the toxic agent required to produce these fatal injuries is about 1,000 times that which produces a temporary inhibition of mitosis. Ultraviolet radiation in adequate dosage results in karyorrhexis of very great numbers of cells in all layers of the corneal epithelium. Even the superficial cells which are normally devoid of mitotic activity undergo karyorrhexis after exposure to ultraviolet radiation. The action spectrum for this effect has a broad peak in the region of 280 mμ (17). Karyorrhexis in the corneal epithelium shows many morphologic similarities to normal mitosis, including a marked increase in the amount of Feulgen positive.
intracellular material. If it is to be regarded as a pathologic and fatal attempt at mitosis, then ultraviolet radiation must be capable of stimulating the senescent cells of the superficial layers in this tissue to engage in this youthful and, for them, fatal activity. Nucleic acid phosphatase is not inhibited at dosage levels of nitrogen mustard, ultraviolet, or x-radiation which cause marked inhibition of mitosis with or without karyorrhexis (22).

Wound healing.—Much of the work on the healing of corneal wounds has been directed toward practicalities of ocular therapeutics (3, 10). Local anesthetics have a markedly inhibiting effect (25). Leopold and Steele (32, 33) found that even the blandest ointment bases caused slight delay in the healing of denuded areas in the cornea. Their findings have been confirmed by others (98, 39). Friedenwald and Heerema (23) found that the toxic factor in lanolin, a component of most ophthalmic ointments, could be removed by washing the lanolin in phosphate buffer. The toxic factor has not been isolated but might be worthy of further study, for there are some analogies between the invasiveness of certain tumors and the healing of epithelial wounds.

Since the work of Oppel (35) and Peters (36), it has been clear that epithelial wounds heal primarily by the migration of adjacent cells into the denuded area and that cellular multiplication plays only a later and secondary role. The truth of this general rule has been abundantly demonstrated with respect to the corneal epithelium. Mechanical injury actually causes a temporary diminution of mitotic activity (12). If the wounds are very small (pin pricks) they may heal completely during the period of mitotic inhibition. Even with larger wounds there is, in general, no net excess of mitotic activity during or following the healing process. The deficit in cells appears to be made up by a diminished rate of desquamation (14). Smelser and Ozanics, however, found some net excess of mitotic activity during the healing of small thermal burns of the corneal epithelium (40).

Following pin-prick wounds of the rat’s corneal epithelium (14), there is a lag period of somewhat more than an hour before the healing process visibly begins. During the second hour after the injury, the marginal cells begin to extend pseudopods into the denuded region and become elongated radially toward the defect. The morphology of these events was studied by Friedenwald, Buschke, and Crowell (16) and later in greater detail by Buschke (1, 2) who has analyzed the separate roles of individual cell movements and of movements of the epithelial syncytium as a unit. The healing of corneal wounds can be studied in vitro as well as in vivo. It is inhibited by anoxia or by cyanide poisoning but not by fluoride. The capacity of cells to migrate can persist when many other vital phenomena have disappeared. Buschke (2) finds that rat corneas, stored in the cold until the basophilic staining capacity of their epithelial cell nuclei has been completely lost, still show healing of pin-prick wounds at a normal rate when incubated at 37° C. following injury.

The events which occur during the lag period immediately following the injury are of special interest, for it is during this period that the stimulus to cellular migration becomes manifest. Friedenwald, Buschke, and Crowell (16) found that during the lag period an exudate is formed within the pin-prick wound. The formation of this exudate is inhibited on incubation at low temperature or in the absence of oxygen. It is therefore the product of active metabolism. One component of this exudate is extractable with lipoid solvents and stainable with silver. Further studies by Buschke (1, 2) show that this exudate is derived at least in part from the sequestrum of fatally damaged cells which must be sloughed off before the healing can begin. The development of the exudate, therefore, may be merely a step in the process of sloughing and not an active stimulus to subsequent healing.

Growth pressure and metaplasia.—In the healing of wounds of the corneal epithelium which extend to the corneal margin, conjunctival as well as corneal epithelium may participate in covering the defect. In darkly pigmented animals there is generally a line of pigmented cells at the corneal-conjunctival border, and the participation of the conjunctival epithelium in the healing process becomes grossly visible through the shifting onto the cornea of this demarcation line. This phenomenon was first described by Mann and Pullinger (34) in their study of mustard gas burns of the cornea. When the corneal stroma has also been injured and is healed with the formation of a vascularized scar, epithelium of conjunctival type may persist as a peninsula covering the vascularized area. In the absence of such a vascularized scar, the demarcation line slowly returns to its normal position.

This interesting phenomenon has been recently reinvestigated by Friedenwald (11). If the whole corneal epithelium of a rabbit is removed, healing takes place entirely from cells of conjunctival origin. During the first week of healing these cells form a single thin layer extending onto and finally covering the whole cornea. During this phase these
cells contain neither mucous globules that are characteristic of the conjunctival epithelium nor glycogen that is characteristic of normal corneal epithelium. About 2 weeks after the denudation the cells assume the characteristic appearance of conjunctival epithelium, forming a membrane two cells deep, with many goblet cells containing mucin. In the course of the succeeding weeks the epithelial layer is gradually transformed into corneal type, forming a layer four to six cells deep with glycogen that is characteristic of normal corneal epithelium. Mitosis counts indicate that the transformation is complete when the conjunctival cells have in their new environment for two to four generations. In those instances in which infected ulcerations of the cornea occurred during the period of denudation, epithelium of conjunctival type persists over the area of the vascularized scar.

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