CELLULAR PROLIFERATION AND REGENERATION FOLLOWING TISSUE DAMAGE

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FINAL REPORT

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ABSTRACT

The main objective is to elucidate the mechanisms which control cellular proliferation during wound healing in tissues of the eye. The effects of chemical and physical injuries on the initiation of the cell cycle in the rabbit lens and cornea have been studied. This was extended to a study of new vessel formation (neovascularization) following injury. The rabbit ocular lens in organ culture was used as an in vitro model system for studies on wound healing. The action of mitogenic agents (e.g. serum, insulin) on macromolecular synthesis (RNA, protein, DNA) and ultrastructure (T.E.M., S.E.M.) were analyzed in the cultured lens. Three subphases between explantation of the lens into a serum-containing medium, and the initiation of DNA synthesis have been described and characterized. In the sequence of ultrastructural and macromolecular changes which have been observed, changes in the cell membranes and cell-to-cell relationships were the earliest. Changes in cell communication (uncoupling) have been suggested as primary events in triggering the cell cycle. Therefore, we have studied the organization of the junctional complexes (notably the gap junctions) in normal tissues, and in tissues at different times after injury in vivo, or exposure to mitogenic agents in vitro. Junctional complexes were studied with the use of replicas of freeze-etched specimens and thin sections for T.E.M. In addition, the regeneration of corneal epithelium and endothelium was analyzed, using newly-developed preparative techniques for scanning electron microscopy. Also, the stimulation of the growth of new vessels (neovascularization) in ocular tissues was studied, using some of the procedures and approaches developed with the ocular lens. Neovascularization is a leading cause of blindness.

Also, emphasis was placed on the development of in vivo and in vitro systems for the testing of potentially-toxic compounds involved in the processing of fossil fuels. This included in vivo studies on the cornea and lens, as well as studies on the lens and other ocular tissues in organ culture.

More recently, a major effort has been devoted to a study of the early stages of lens fiber cell degeneration which lead to cataract formation in humans. Procedures have been worked out in our laboratory for the ultrastructural and elemental analysis of specific human lens opacities, localized by the stereo-photographic procedures developed by the Cooperative Cataract Research Group (Chylack, L.T. Jr., Invest. Ophthal. 96:888-892, 1978). In this study, the techniques of Energy Dispersive X-ray Analysis (EDXA) were used in combination with scanning electron microscopy.

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The work in our laboratory, supported by D.O.E. Contract DE-AC02-76EV02401, has been very productive. There have been 46 publications on this contract thus far, and two major papers, based on work done during the contract period are presently being written.

The main thrust of the program has been a study of the lens and cornea during wound healing, and certain disease states. A major emphasis has been on the human. Fresh eye tissues from the operating rooms, located in our building, and also tissues from the Michigan Eye Bank, which is located in our department, the Kresge Eye Institute, have been used.

A list of publications issued during the total agreement period is included in this report, plus most of the reprints published since the last report.

Using techniques developed at the Howe Laboratory of Ophthalmology, Harvard University (Chylack, L.T., Jr., Invest. Ophthalm. 96:888-892, 1978), we have been able to localize specific opacities in cataractous human lenses for subsequent analysis with scanning electron microscopy, transmission electron microscopy, and elemental analysis with energy dispersive X-ray analysis techniques (EDXA).

A type of opacity, found in human cataractous lenses, has been found to be characterized by a high ratio of phosphorus to sulfur by EDXA (40). These opacities were also found to contain an accumulation

of unit membranes. These results will be submitted for publication shortly.

In a related study (35), possible correlations between the density of an opacity and its ultrastructure were presented and discussed. Details of the work performed on cornea and lens since the last report are presented in the enclosed reprints. These experiments are not, therefore, repeated here. (This is according to the statement in the Guide for Reporting of Scientific and Technical Information (p.3): "Reprints of all such material not previously submitted shall be appended and material contained therein need not be duplicated in the report".).

LIST OF PUBLICATIONS ISSUED DURING TOTAL AGREEMENT PERIOD

1. Harding, C.V., Harding, D., Peters, V., Kuwabara, T., Reddan, J., Unakar, N., Bagchi, M., Schnur, T. and Gordon, S.: Epithelial cell surfaces and the basement membrane in the ocular lens, studied with the scanning electron microscope, *Exptl. Eye Res.* 16:1-7, 1973.
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- Harding, C.V., Susan, S. and Cohn, E.: Unit membrane redundancy in spherical structures within the ocular lens, *Ophthal. Res.* 10: 7-15, 1978.
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