Cellular Proliferation and Regeneration Following Tissue Damage
Contract # EY-76-S-02-2401 Kresge Eye Institute of Wayne State University
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PROGRESS REPORT

That portion of our program which deals most directly with the development and use of short-term bioassays for potentially toxic agents, involves in vivo and in vitro systems.

Work on cornea in vivo: Since the cornea is the anterior-most transparent structure of the eye, it is particularly susceptible to injury. Mrs. Hedwig Murphy, a graduate student in my laboratory, but working in collaboration with Dr. Richard Thoft (Retina Foundation, Boston) on this project, has completed a study of the role of wounding the ocular surface epithelium and the role of leukocytes in neovascularization in the cornea. Neovascularization in the cornea or retina is a leading cause of irreparable blindness.

In vivo studies on the corneal endothelium have also been done (by S. Gordon), in which he has characterized the wound healing process with scanning electron microscopy.

Tissue culture studies are now being carried out in four separate laboratories within K.E.I. In our laboratory, we have concentrated on culture of the rabbit retinal vasculature. Time-lapse photography autoradiography, E.M. (T.E.M. and S.E.M.) and freeze-etch techniques (all now established in our lab) are being applied. The whole ocular lens of the rabbit in organ culture has been well established in our lab and we are now starting to use the human lens. We feel the above systems can be used for testing the effects of potentially-toxic agents.

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Freeze-etch set up

The Balzers 301 freeze-etch unit with all ancillary equipment has been set up and is now in routine use. This major equipment purchase was made possible through funds provided by The Kresge Foundation. Freeze-etch E.M. studies of lens epithelium and lens fiber cells have been initiated. Gap junctions have been identified in both epithelium and lens fiber cells. Further analysis of junctional complexes in the normal lens are necessary before a study of possible changes in the junctional complexes during development, wound healing, and cataractogenesis are possible. The availability of this equipment is now making it possible for other studies on other ocular tissues such as in Dr. Essner's studies on the retinal pigment epithelium and outer segments.

A study of gap junctions of chick lens fiber cells has now been completed, and published in Experimental Eye Research, (Kuszak, Maisel and Harding). The essential findings in this study demonstrate that gap junctions can be identified by scanning electron microscopy (S.E.M.), and that they comprise very extensive areas of the plasma membrane of chick lens cortical fiber cells.

The evidence for this is as follows: Scanning electron microscopy of the surface of cortical fiber cells revealed many oval and circular surface plaques varying in area from $0.12 \mu\text{m}^2$ to $13.1 \mu\text{m}^2$. The plaques were found on both wide longitudinal surfaces of the hexagonal fibers. Freeze-fracture analysis of the fiber cells revealed numerous oval and circular gap junctions ranging in area from $0.02 \mu\text{m}^2$ to $12.5 \mu\text{m}^2$. This close correlation in size between the plaques as seen by S.E.M. and the gap junctions as noted by freeze-fracture indicate that the plaques seen by S.E.M. represent the gap junctions found by freeze-fracture analysis.

Scanning electron microscopy

A study of the structure of the normal rabbit lens emphasizing the use of scanning electron microscopy has been completed and published (Ophthal. Res. 8, 1976). This is to serve as the basis for further studies on wound healing and cataract development. The unique properties of the rabbit lens as outlined in that publication, make it particularly suitable for certain kinds of studies involving S.E.M.

Spherical structures found in the lens under certain abnormal circumstances including human cataracts

'Spherical structures', found in the nuclear region of some rabbit lenses have been observed with scanning electron microscopy (S.E.M.) and transmission electron microscopy (T.E.M.). Their surface, as seen with S.E.M., is similar to that of fiber cells normally seen in this region of the lens. However, T.E.M. reveals that multiple unit membranes (2-10) make up their surface. A description of these structures has recently been published (Harding, Susan, Jampel and Cohn, 1978). Evidence for the possible mode of origin of this membrane redundancy is presented and discussed. Comparisons and contrasts are made with globular structures (possibly Morgagnian bodies) seen in human cataractous lenses. Differences between these 'spherical structures': seen in the rabbit, and 'globular structures' seen in human cataractous lenses are discussed.

N.E.I. Cooperative Cataract Research Group

Our laboratory is now set up to obtain human cataractous lenses, which can be photographed within minutes after surgery, and then immediately prepared for one of a number of different kinds of study (e.g., fixing for electron microscopy; rapid freezing for biochemical analysis). These lenses are then sent to other laboratories for study, or utilized in our own laboratory.

Our closest collaboration in this program is with Dr. Venkat Reddy at the Institute of Biological Sciences, Oakland University, and Dr. Leo Chylack of Harvard Medical School. Dr. Chylack has visited our laboratory, and checked over our equipment so that our photographic standards will be comparable with those of the other laboratories involved in the N.E.I., Cooperative Cataract Research Group (CCRG).

Our own contribution to the CCRG program, in addition to supplying lenses classified under standard conditions, will be the study of human cataracts utilizing transmission and scanning electron microscopy, combined with energy dispersive x-ray analysis, in addition to freeze-etch electron microscopy. Energy dispersive x-ray analysis of human cataracts has shown distinct differences in the content of phosphorus and sulfur between intact fiber cells and material which we believe to be derived from inter-cellular spaces (or water clefts). (Harding, Susan, Worgul, Solway and Maser, 1978).

Newborn rabbit retinal vasculature in tissue culture

Techniques for the growth of new-born rabbit vasculature in tissue culture have been further worked out. Techniques that involve the use of collagen coated cover-slips in addition to the use of plasma to maintain the vascular spread out in culture have been established. Time-lapse photography of the isolated vessels is now in routine use. Also a preliminary study of the incorporation of tritiated thymidine by the cells in these isolated vessels at various times after being established in tissue culture is well under-way. Thus far the incorporation of thymidine in vessel cells has been detected following periods of a week in culture. The effects of growth factors such as Volkman's tumor growth factor will now be studied.

Studies on human corneal endothelium in primary graft rejects

Eye bank corneas which were used for transplantation and then subsequently underwent primary graft failure were used in a study of the scanning electron microscopy of their endothelial surface. These human primary graft failures showed a variety of cellular forms and cellular interrelationships on their endothelial surfaces. The main results of this study were reported at the A.R.V.O. Spring meeting, 1977.

A more extensive study was presented at the XXIII International Congress of Ophthalmology in Kyoto, Japan, in May 1978, and is now in press (Cowden and Harding, 1978).

Corneal neovascularization following injury

Mrs. Hedwig Murphy, a graduate student working toward her Ph.D. in our department, has been concentrating her efforts primarily on the study of neovascularization in the rabbit cornea following injury. With partial support from our grant, she has carried out experiments on injury-induced neovascularization in the rabbit cornea in the laboratories of Drs. Claes Dohlman and Richard Thoft at Harvard Medical School. This investigation was planned and is being carried out in conjunction with our proposed study of induced vessel growth as outlined in the original proposal. Significant progress has been made in the study of injury induced thymidine incorporation in blood vessels of the limbal area and correlated permeability changes in these vessels about to undergo neovascularization.

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