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The Mammalian Iris-Ciliary Complex Affects Organization and Synthesis of Cytoskeletal Proteins of Organ and Tissue Cultured Lens Epithelial Cells

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Abstract A water soluble growth inhibitor was isolated from the mammalian ocular iris-ciliary complex. The molecular weight of this protein is 10 kD or lower as determined by ultrafiltration fractionation. The iris-ciliary (IC) complex water soluble protein(s) significantly inhibits synthesis of lower molecular weight proteins of the epithelial cells of the organ cultured mammalian ocular lens. It was also found that this inhibitory effect of IC is mediated via the structural organization of the lens. Monolayer cultures of the lens epithelial cells exposed to IC did not manifest any inhibition of their protein synthesis. Moreover, these tissue cultured lens epithelial (TCLE) cells showed a significant increase in their protein synthetic activities in response to the presence of IC factors in the culture medium. It is postulated that the IC activity is modulated via either the lens capsule, an extracellular matrix, or due to the specific organization of the intact lens.

The specific effects of IC on the cytoskeletal organization and synthesis in the organ cultured lens epithelial (OCLE) and TCLE cells were also examined. Both groups, treated with IC factors, manifested significant alterations in their protein synthetic activities and cytoskeletal architecture. The ^{3}H-leucine incorporation experiments showed that α-actin and α-tubulin synthesis is partially inhibited by IC factors in OCLE cells but vimentin synthesis is not, whereas in TCLE cells all of them showed increased synthesis in response to IC factors. Turnover rates of these proteins in both OCLE and TCLE cells were also computed. The immunofluorescence and microscopic evaluation of OCLE and TCLE cells exposed to IC factors illustrated significant alteration in the cytoarchitecture of the filaments. We demonstrate that an inhibitor(s) molecule of 10 kD or lower size isolated from IC inhibited protein synthesis of OCLE cells and stimulated protein synthesis in TCLE cells. The IC factor also affects the synthesis and organization of cytoskeletal filaments of both the OCLE and TCLE cells.

Key words: iris-ciliary complex, ocular lens, vimentin, tubulin, actin, organ culture, tissue culture, protein synthesis, growth inhibition

Previously we and others have reported that mammalian iris-ciliary complex effectively inhibits its protein synthesis and increases sodium content of the organ cultured ocular lens epithelial and fiber cells [Owers and Duncan, 1979; Bagchi et al., 1987a, 1988]. The IC displays this activity whether it is attached to the lens or co-cultured with it [Bagchi et al., 1988]. It was also found that a water soluble extract of fresh IC retains this inhibitory property. The IC extract inhibited protein synthesis by both the epithelial cells and fiber cells of the organ cultured lens [Bagchi et al., 1988].

Mammalian IC is highly vascularized [Clemente, 1985; Niyogi et al., 1991] and it was found that the organ culture of IC is very difficult as it manifests necrosis even in the presence of 20% rabbit serum [Niyogi et al., 1991]. Thus, many observed effects of IC on intact lens could be imparted by degenerating IC [Rothstein et al., 1972]. It is also possible that IC cells contain growth inhibitors and during necrosis of these cells, massive amounts of growth inhibitors are released in the medium, causing observed effect on the lens cells [Niyogi et al., 1991]. This is probable as water soluble extract of the IC contains growth inhibitory activity. To purify and characterize growth inhibitor molecule(s) of the IC, it was fractionated by ultrafiltration and a smaller than 10 kD fraction retained most of the growth inhibitory activity. Effect of this inhibitor was also tested on the tissue cultured lens epithelial cells. These experiments showed that

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IC has no growth inhibitory effect on the tissue cultured lens epithelial cells. However, IC factors induced specific morphological changes in the tissue cultured lens epithelial cells.

Inhibitory factor purification experiments demonstrated sequestration of inhibitory molecules in a 10 kD or less fraction. Slab gel electrophoresis of in vitro phosphorylated proteins of IC presented a prominent 4 kD polypeptide. This polypeptide is synthesized by pigmented epithelial cells of the IC. This phosphoprotein is also present in aqueous humor but not in vitreous humor [Banerjee et al., 1991a]. However, whether this is the inhibitor molecule is not yet clearly established.

Here we report the effects of IC factor on cytoskeletal proteins (actin, vimentin, and tubulin) by electron microscopy, radioactive labeling, and Western blot analyses.

METHODS

Organ Culture

Rabbit lenses were obtained from freshly killed New Zealand white rabbits of about 2 kg body weight. The culture medium was TC-199 at pH 7.4 and an osmolarity of 310 mOsm. The lenses were cultured in a water bath at 33 ± 1°C [Bagchi et al., 1988].

Tissue Culture

Rabbit lens epithelial cells were grown in DMEM with 20% rabbit serum at 37°C with 5% CO_2. The cells were plated in flasks or on coverslips for experimentation after their second passage.

Iris-Ciliary Body Water Soluble Factors

Iris-ciliary bodies from rabbit eyes were excised, washed in PBS, and then disrupted in ice-cold 40 mM MOPS (pH 7.0), 5 mM $MgSO_4$, 1 mM DTT, and 1 mM EGTA, with a low clearance potter-elvehjem homogenizer. It was spun in an Eppendorf centrifuge at 14,000 rpm for 10 min at 4°C and the supernatant (whole IC factor) was collected for further fractionation by filtering it through a 10 kD molecular weight cut off Centriprep (Amicon, Beverly, MA) filter. The residuum and the filtrate were used separately. In all experiments, IC whole protein and residuum were used at 200 μg/ml culture media. Filtrate proteins were used at 20 μg/ml. The IC proteins were fractionated by the Amicon ultrafiltration units as it gave a much better recovery of the 10 kD or smaller fraction compared with chromatographic fractionation on a column of Sephacryl S-100 (Pharmacia-LKB, Piscataway, NJ) as used by us for fractionating vitreous humor proteins [Banerjee et al., 1991b]. Both the filtrate of ultracentrifugation and the residuum were used to assure if there were more than one inhibitory factor present in the total IC. However, according to the manufacturer's specification, the residuum retains about 10% of the smaller than 10 kD molecules; thus, any observed activity could be due to small molecular weight VH proteins in this fraction.

Protein Synthesis and Quantitation

In some experiments, 6 h prior to completion, lenses/cultures were pulsed with ^{3}H-leucine (Amersham, Arlington Heights, IL) at a concentration of 50 μCi/ml in leucine free RPMI-1640 culture medium (with or without iris-ciliary body factors). It should be noted that the cells were exposed to the factors all along the 24 h incubation period. Following the pulse, epithelial cell proteins were analyzed by SDS-PAGE [Laemmli, 1970] and either stained with Coomassie blue R-250 or transferred to nitrocellulose using a Biorad (Richmond, CA) electroblotting apparatus according to the manufacturer's instruction onto 0.05 μm pore size nitrocellulose (Schleicher & Schuell, Dassel, Germany) for Western blotting or fluorographed [Bonner and Laskey, 1974]. For the organ culture experiments, epithelial proteins from a single lens were divided into four parts. They were used in the gel electrophoresis and fluorography, as well as in Western blotting for immunolocalization and radioactivity measurement of vimentin, α-tubulin, and α-actin. Similarly, for the tissue culture experiments protein from each control or treated flask was also divided into four parts and used as above. Four sets of experiments were done which showed similar pattern in results. Data from one such set is presented in this paper as statistical treatment could not be applied on the data from all four sets due the variation in the amount of radioactivity incorporated into proteins from set to set.

The stained gels, fluorograms, and the Western blots (horseradish peroxidase stained) were scanned by an AMBIS (San Diego, CA) optical imaging system for quantitation. Images were captured by a 732 × 480 pixel digital CCD video camera mounted above a light box which provided both reflected and transmitted light as needed. After data acquisition, images were stored as files to permit further analysis and comparison by the AMBIS Optical Imaging soft-

ware version 2.1 and a Microsoft mouse on an AT-compatible 386 SX computer. The data were obtained from the sample bands by the multiple shapes method. A mouse was used to draw extraction enclosures around the bands under study. A sample background was subtracted from the bands for quantitation. After the bands were quantitated, they were cut out with surgical scissors and the nitrocellulose filter dissolved in DMSO and counted for ^{3}H-leucine dpm by liquid scintillation spectrometry.

Fine Structure Analysis

Fresh rabbit lenses were organ cultured with or without iris-ciliary body for 24 h. At the end, lenses were fixed in cold buffered glutaraldehyde [Bagchi et al., 1985]. The anterior capsule epithelium was post fixed in 2% OsO_4. The tissues were stained en bloc with 2% uranyl acetate. Thin sections were counter stained with lead citrate and viewed with a JEOL 100A TEM at 60 kV.

Immunofluorescence

Organ cultured rabbit lenses and lens cells on coverslips were incubated with whole iris-ciliary body factor for 24 h. Thereafter, they were washed in PBS three times and fixed in 3.7% formaldehyde in PBS for 1 h and then washed again with PBS several times. Immunostaining for cytoskeletal structures was done by monoclonal antibodies against vimentin (#V9), α-tubulin (#DM1A), and α-actin (#1A4) obtained from Sigma. The secondary antibody was a rab-

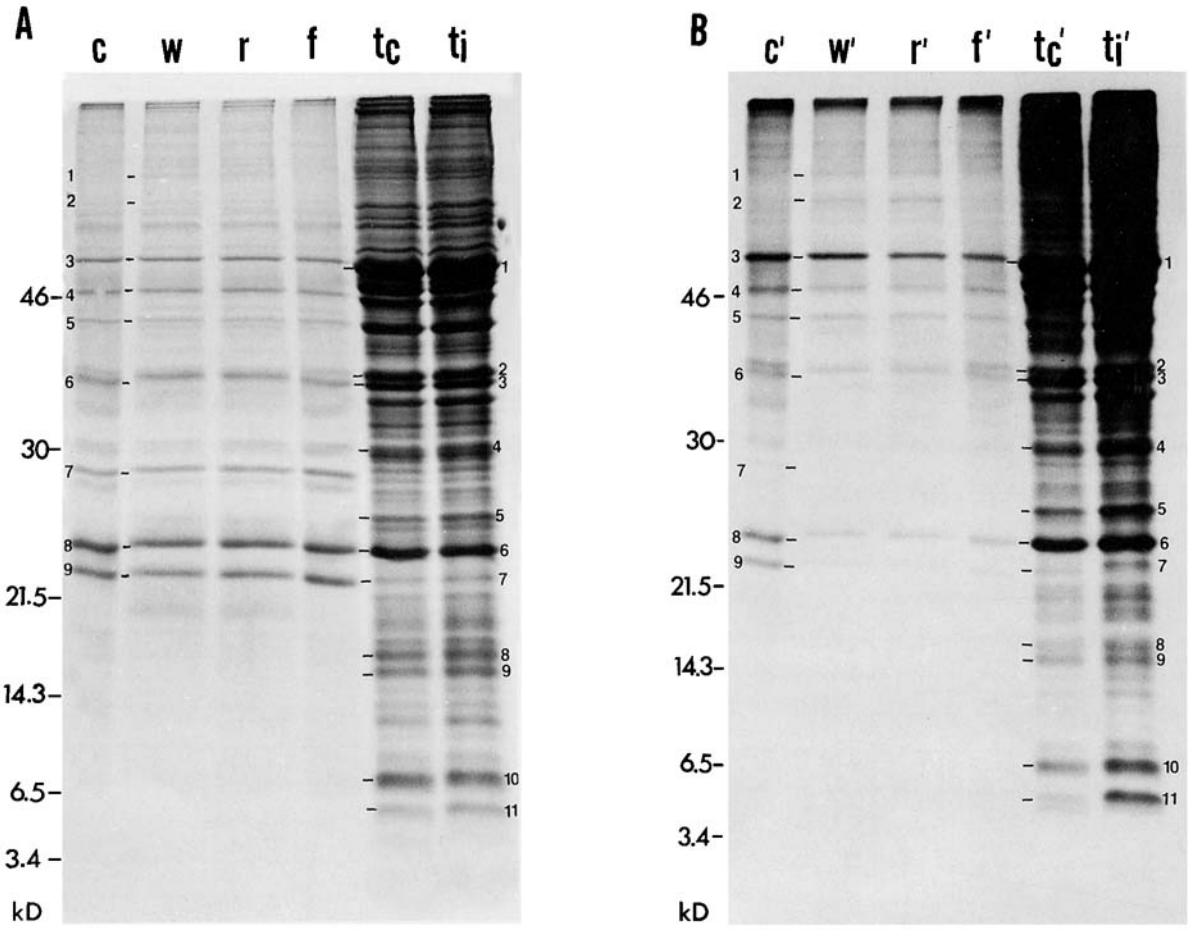

Fig. 1. Depression of protein synthesis in organ cultured lens epithelial cells and elevation of protein synthesis in tissue cultured lens epithelial cells upon treatment with iris-ciliary body factors. **A:** Coomassie brilliant blue R-250 stained gel. OCLE cell proteins were loaded at 100 μg and TCLE cell proteins were loaded at 300 μg total protein per slot. c, proteins from control OCLE cells without treatment; w, proteins from OCLE cells treated with whole IC factors without fractionation; r, proteins from OCLE cells treated with the residuum fraction of IC after filtration through a 10 kD MWCO Centriprep; f, proteins from OCLE cells treated with the filtrate fraction of IC; t_c, proteins from control TCLE cells without treatment; t_i, proteins from TCLE cells treated with whole IC factors without fractionation. **B:** Fluorograph of the gel shown in A. The lanes here are marked with primed characters.

bit FITC-IgG against mouse IgG (Sigma) [Banerjee et al., 1991a]. Tissues were mounted in 90% glycerol in PBS and viewed with a Zeiss Axiophot microscope.

RESULTS

Figure 1 displays gel profile (A) and fluorogram of ^{3}H-leucine pulsed proteins (B) isolated from the epithelial cells of lenses organ cultured in TC-199 or TC-199 supplemented with total IC (200 μg/ml), 10 kD filtrate IC fraction (20 μg/ml), or residuum IC fraction (200 μg/ml) and gel profile and fluorogram of proteins isolated from tissue cultured lens epithelial cells maintained in RPM1-1640 or RPM1-1640 supplemented with total IC fraction (200 μg/ml). This figure clearly demonstrates that the dye stained gel profiles of proteins obtained from epithelial cells of lenses cultured with IC factor(s) are similar, both in intensity of dye in each band and banding pattern. The protein profiles of TCLE cells treated with total IC factor is similar to untreated cells. However, the protein profile of TCLE cells is distinctly different from that of epithelial cells of organ cultured lenses. Figure 1B is the fluorogram of Figure 1A. It is evident from the fluorogram that the protein synthetic activity of epithelial

TABLE I. Quantitative Analysis of Radioactivity per Arbitrary Unit of Gel Band Stain as Calculated by AMBIS System*

Protein band no.		Epithelial (LE) cells of organ cultured lens				Tissue cultured LE cells	
		Control	Whole IC extract treated	Residuum IC extract treated	Filtrate IC extract treated	Control	Whole IC extract treated
	G	42,783	52,512	54,723	52,050	27,878	27,625
1	F	7,754	7,474	7,345	7,297	30,344	38,991
	F/G	*0.18*	*0.14*	*0.13*	*0.14*	*1.09*	*1.41*
	G	61,709	65,463	63,890	55,183	14,783	14,977
2	F	6,329	5,185	5,331	4,011	21,105	27,480
	F/G	*0.10*	*0.07*	*0.08*	*0.07*	*1.43*	*1.83*
	G	62,508	78,148	74,752	73,492	25,432	26,392
3	F	15,278	19,600	18,462	16,127	18,283	26,734
	F/G	*0.24*	*0.25*	*0.25*	*0.22*	*0.72*	*1.01*
	G	59,701	67,373	63,990	62,408	32,970	31,793
4	F	10,398	8,730	8,391	8,361	17,390	26,490
	F/G	*0.17*	*0.13*	*0.13*	*0.13*	*0.53*	*0.83*
	G	50,836	59,754	60,074	68,216	30,760	28,422
5	F	7,625	8,639	7,080	7,889	14,557	24,558
	F/G	*0.15*	*0.14*	*0.12*	*0.12*	*0.47*	*0.86*
	G	55,722	91,835	89,181	58,275	40,544	39,560
6	F	7,456	5,841	5,405	6,427	19,707	28,655
	F/G	*0.13*	*0.06*	*0.06*	*0.11*	*0.49*	*0.72*
	G	73,079	94,666	96,418	72,928	53,934	50,340
7	F	3,675	2,500	2,167	2,333	6,140	11,857
	F/G	*0.05*	*0.03*	*0.02*	*0.03*	*0.11*	*0.24*
	G	56,806	82,798	70,067	62,700	45,162	33,576
8	F	8,977	3,833	3,073	3,702	7,052	11,768
	F/G	*0.16*	*0.05*	*0.04*	*0.06*	*0.16*	*0.35*
	G	71,443	109,107	98,790	76,043	43,483	37,678
9	F	6,818	2,094	1,036	2,055	7,762	12,343
	F/G	*0.10*	*0.02*	*0.01*	*0.03*	*0.18*	*0.33*
	G					65,950	69,152
10	F					12,157	21,274
	F/G					*0.18*	*0.31*
	G					89,120	86,494
11	F					8,077	23,624
	F/G					*0.09*	*0.27*

*F = fluorograph of G; G = gel band stained with Coomassie blue. Both the amount of dye and silver precipitate were measured by AMBIS system. Band numbers and treatment groups are same as in Figure 1.

cells of lenses organ cultured with IC factors is significantly depressed. Moreover, synthesis of all proteins is not uniformly diminished (see Table I). Figure 1B also demonstrates that the total IC factor stimulates protein synthesis by the TCLE cells. This stimulatory activity is more prominent in the low molecular weight proteins of the TCLE cells (Fig. 1B).

Table I shows the comparative amount of dye and silver grains in gel bands and fluorogram bands measured by the AMBIS system. These data illustrate that specific bands have similar amounts of protein but different amounts of radioactive leucine, thus indicating different specific activity of radioactive labeling of proteins. In general, lower molecular weight polypeptides of the epithelial cells of the organ cultured lenses showed greater inhibition of synthesis by IC. The TCLE cells on the other hand displayed increased protein synthesis in response to IC. Virtually all marker proteins showed greater rate of synthesis; the lower molecular weight polypeptides depicted relatively higher increase in the rate of synthesis compared to higher molecular weight polypeptides.

Figure 2 shows the immunoblot of vimentin, α-tubulin, and α-actin from epithelial cells of lenses organ cultured with different IC fractions and from TC lens epithelial cells incubated with or without total IC. The blots show that both epithelial cells of organ cultured lenses and TC epithelial cells have significant amounts of vimentin and tubulin. However, α-actin could not be detected in the epithelial cells of organ cultured lenses. TC cells do show presence of α-actin. As mentioned in Methods, area and dye intensity of each blot was digitized to measure relative amount of protein in each band. Each band on the blots was also carefully cut, dissolved in DMSO, and put in scintillation cocktail to measure the amount of radioactivity in them by liquid scintillation spectrometry.

Figure 3 shows the analysis of the data obtained from above mentioned measurements. The relative amounts of vimentin, α-tubulin, and α-actin in the epithelial cells of lenses organ cultured with different IC fractions and tissue cultured cells treated with total IC are shown in Figure 3A. This histogram shows that the amount of vimentin did not change significantly in any of the treated cells, when compared to control. The amount of α-tubulin in the epithelial cells of organ cultured lens treated with 10 kD IC showed appreciable decrease. The α-actin

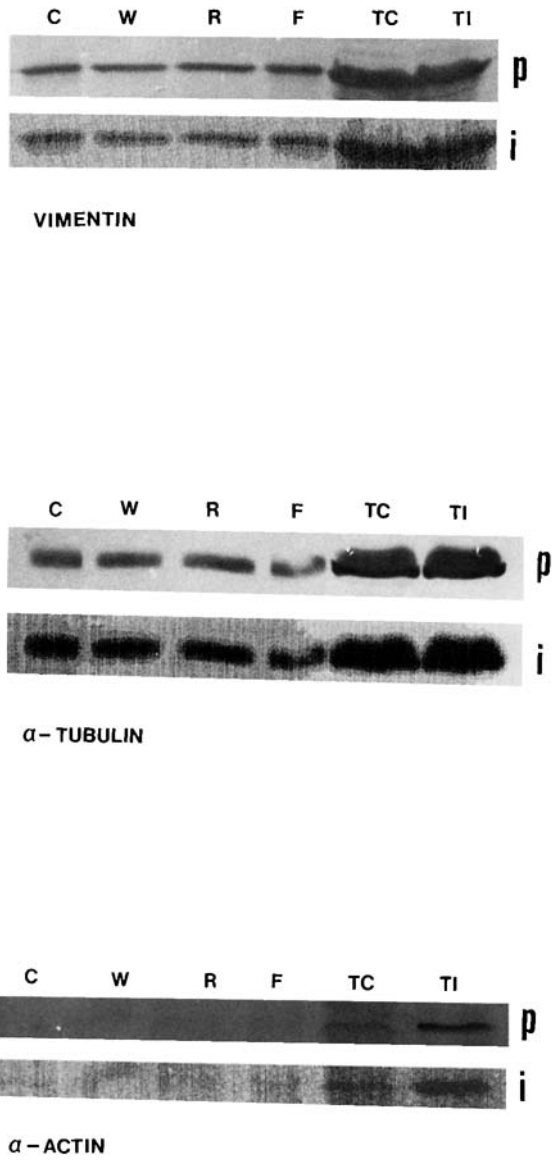

Fig. 2. Western blot with anti-vimentin, –α-tubulin, and –α-actin. The characters corresponding to the lanes mean the same conditions as for Fig. 1A. In each set **p** marks the photograph of the Western blot and **i** the digitized image of the blot used for quantitation.

was measurable only in the tissue cultured cells and showed significant increase in the total IC treated cells. Figure 3B displays the total amount of radioactivity in each band of α-actin, vimentin, and α-tubulin visualized by immunoblot technique. The histogram shows that the amount of radioactivity in vimentin, α-actin, and α-tubulin of the epithelial cells of control organ cultured lenses are relatively higher than the IC treated ones. The IC treatment caused

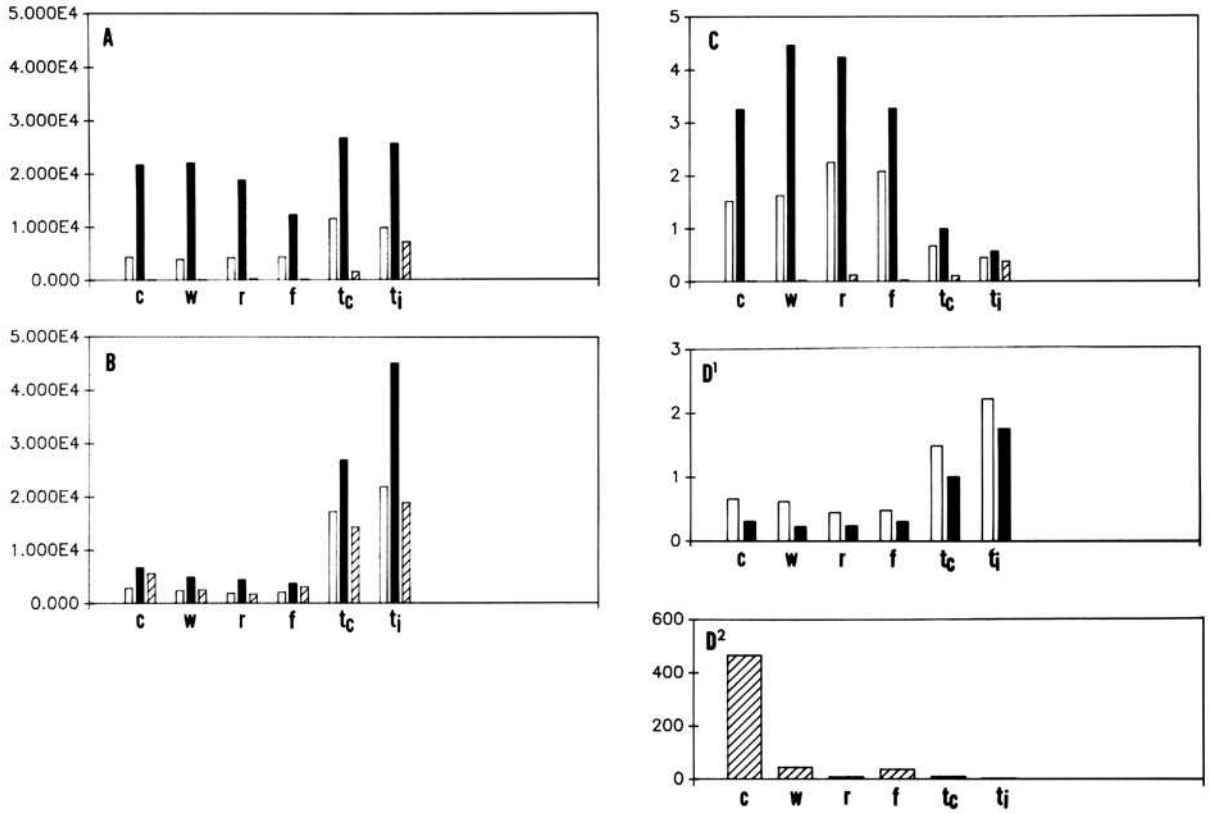

Fig. 3. In this figure the characters corresponding to the bars mean the same conditions as in Fig. 1A. Open bars, vimentin; closed bars, α-tubulin; hatched bars, α-actin. The same Western blots (shown in Fig. 2) were used for measurement in both A and B. **A:** Quantitation of amount (ordinate) of cytoskeletal proteins in the cultured cells in arbitrary units, measured from the Western blots shown in Fig. 2 digitized images. **B:** Plot of ^{3}H-leucine dpm (ordinate) of cytoskeletal proteins in the cultured lens cells. **C:** Ratio of amount/dpm of the cytoskeletal proteins. This can be used as a measure of stability of the proteins in the lens cells under various conditions. **D^1, D^2:** Ratio of dpm/amount of the cytoskeletal proteins. This can be used as a measure of the relative amount of degradation of the three proteins under various conditions.

increased amount of radioactivity in the cytoskeletal filament proteins of TCLE cells.

Figure 3C demonstrates the specific activity of the radioactive filament proteins (dye/dpm). If the half-life of a protein is longer than its rate of breakdown, then the total amount of this protein will increase. This accumulation of protein will also indicate that the protein is relatively stable. The data presented in the histogram (Fig. 3C) indicates that vimentin and α-tubulin of the epithelial cells of lenses treated with various IC fractions are rather stable, with some increase in the stability of vimentin treated with either 10 kD IC or residuum of IC, whereas α-tubulin shows more stability with total IC and residuum IC. In the tissue cultured lens cells, both vimentin and α-tubulin show decreased stability in the presence of total IC but α-actin shows significant increase in its stability.

Figure 3D^1, D^2 (dpm/dye) analyze the rate of degradation of cytoskeletal filamentous proteins. These data are the corollary of Figure 3C. Increased stability indicates decreased breakdown and vice versa. Figure D^1 depicts that the rate of degradation of vimentin and α-tubulin in the organ cultured lens cells did not significantly change in responses to IC factors, whereas in tissue cultured cells vimentin and α-tubulin showed appreciable increase in their degrada-

Fig. 4. Immunofluorescence pictures of lens cells in organ culture. The left panel (**A,C,E**) are control and the right panel (**B,D,F**) are total IC factor treated cells. Anti-vimentin stain is shown in A (distinct perinuclear location) and B (dispersed and diffuse); anti–α-tubulin is shown in C (perinuclear) and D (diffuse); anti–α-actin is shown in E and F. The magnification bar is 20 μm.

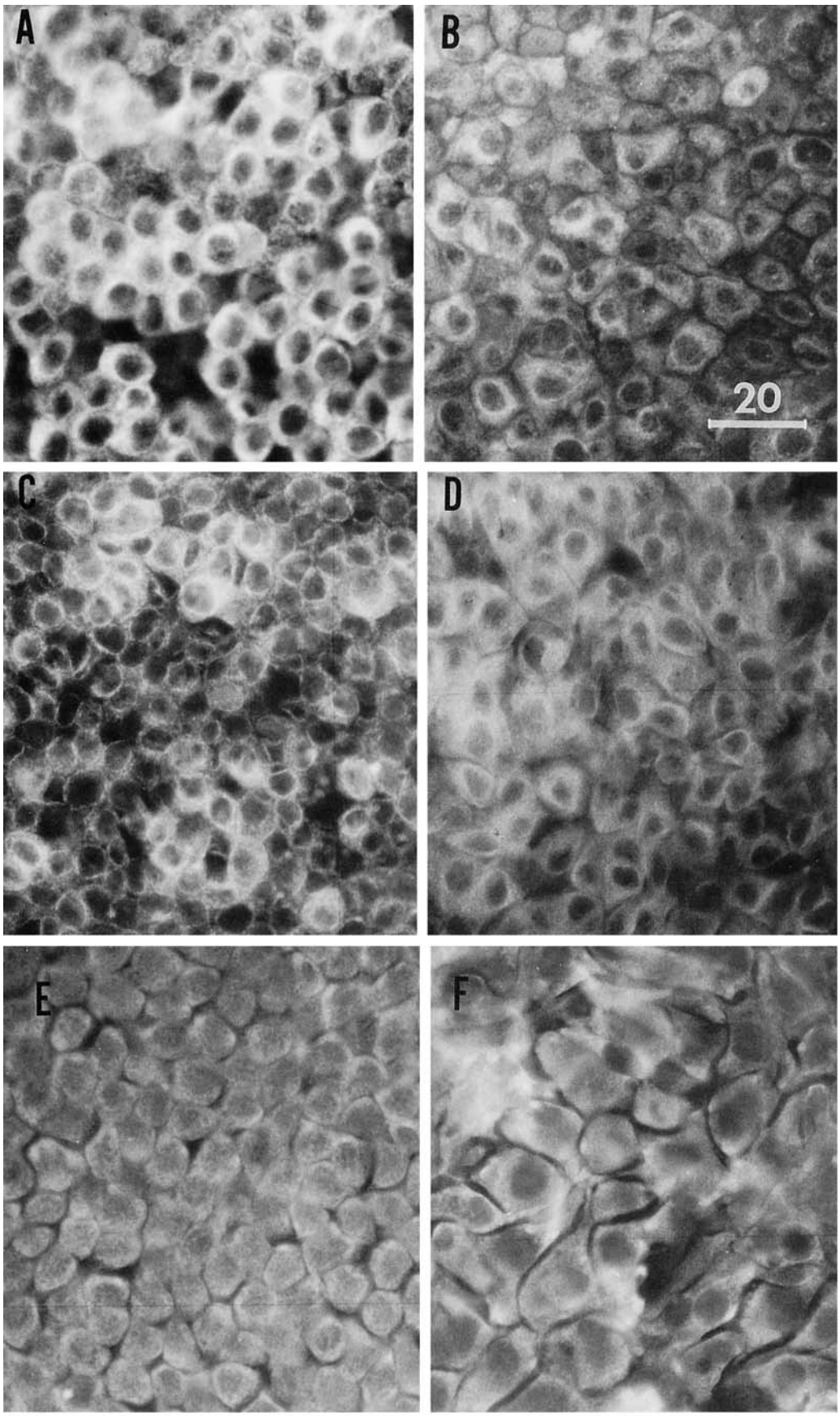

Figure 4.

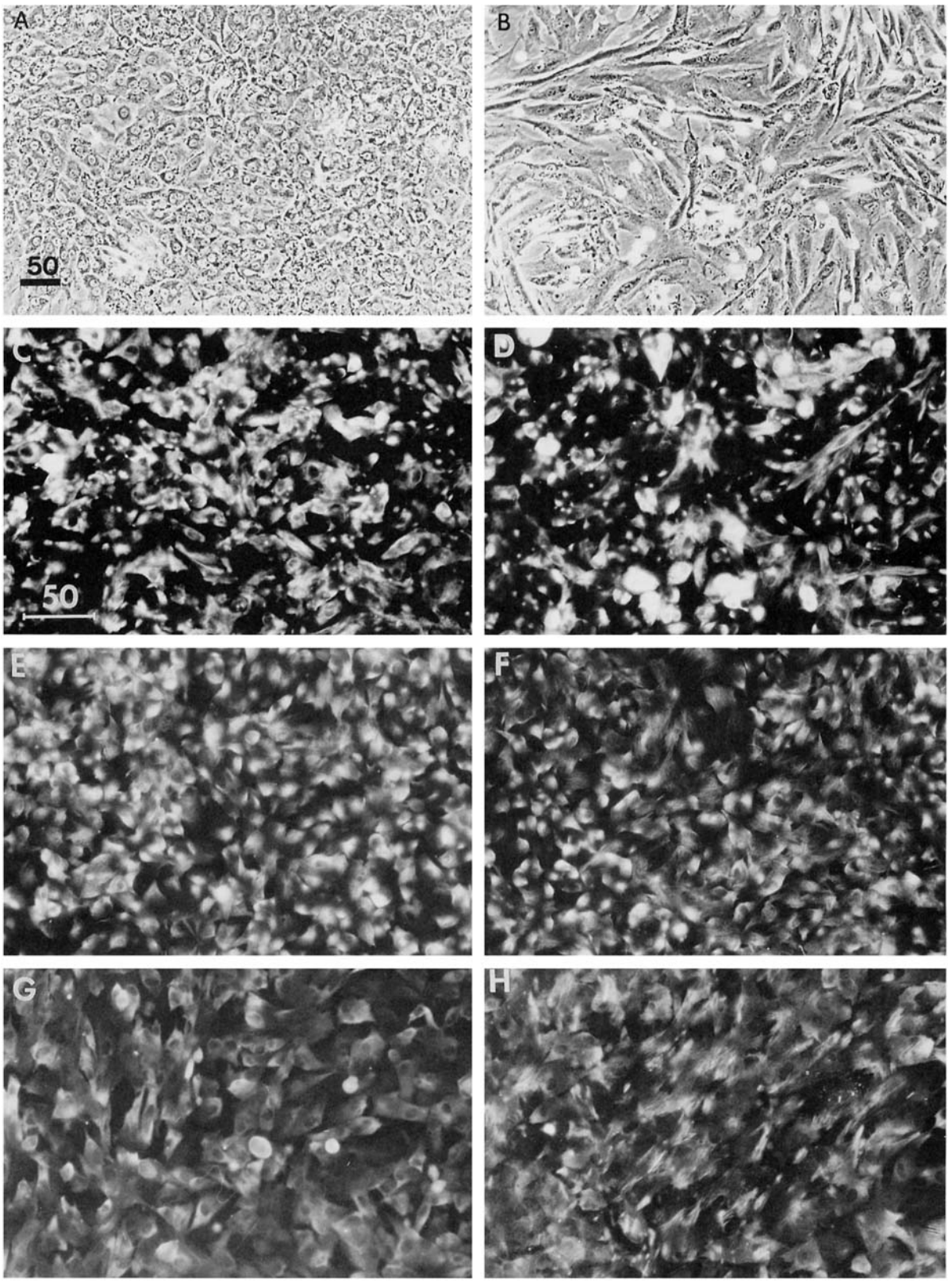

Figure 5.

tion rate when treated with total IC. Figure 3D[2] demonstrates that the α-actin turnover in the epithelial cells of control lenses is extremely high and shows significant decrease in the rate in the presence of IC factors. The α-actin turnover rate of treated tissue cultured lens cells is nearly double that of control TC cells. These results suggest that the IC factor could intefere with the turnover rate of actin filaments of tissue cultured or organ cultured lens epithelial cells.

Figure 4 shows the light micrographs of the epithelial cells of lenses stained with anti-vimentin, –α-tubulin, and –α-actin monoclonal antibodies. Contralateral lenses were cultured with or without total IC (200 μg/ml) for 72 h and then fixed and prepared for antibody labeling. Figure 4A,B shows, respectively, epithelial cells of lenses cultured without and with total freeze dried IC (200 μg/ml) and decorated with fluorescent anti-vimentin antibodies. Control cells show distinct perinulear vimentin, whereas IC treated cells display dispersed and diffused vimentin in their cytoplasm; Figure 4C,D shows control and IC treated epithelial cells of whole lenses decorated with fluorescent anti–α-tubulin antibodies. The control epithelial cells show tubulin perinuclear in location, whereas IC treated cells show distinctly different α-tubulin distribution. Figure 4E,F shows α-actin distribution in the epithelial cells of the organ cultured lenses. These light micrographs clearly demonstrate that the presence of IC in the culture medium significantly increases α-actin accumulation in these cells, whereas α-actin is poorly detectable in the epithelial cells of control lenses.

Figure 5 shows the effect of total IC on the tissue cultured lens epithelial cell morphology and cytoskeletal filaments using fluorescent tagged antibodies. These light micrographs clearly illustrate that the presence of total IC in the culture medium induces cell elongation. Antibody labeling experiments displayed that IC has no direct effect on vimentin distribution as per amount; however, α-tubulin seems to be less detectable in the cells incubated with total IC. As previously shown in Figure 4, the IC has significant effect on α-actin. The IC treated cells have a relatively higher amount of fluorescent dye and abundant stress fibers. It is possible that the presence of stress fibers is related to cell elongation.

Figure 6 shows the electron micrographs of the epithelial cells of lenses cultured for 24 h (A) or 48 h (B) in 10% serum supplemented TC-199 medium. These micrographs clearly demonstrate that epithelial cells of lenses maintained in organ culture retain their normal morphology. Both micrographs display perinuclear intermediate sized filaments, rough endoplasmic reticulum (ER), polysomes, and intact cell and nuclear membrane. When lenses were incubated in presence of IC for 24 h, cytoskeletal filament distribution of the epithelial cells showed distinct disorganization. Figure 7A,B shows two cells taken from central and pre-equatorial regions of the lens, respectively. Both cells show abundant dispersed filaments all over the cytoplasm. Other cytoplasmic morphology seems to be normal (e.g., presence of rough and smooth ER, polysomes, etc.). Figure 8 has electron micrographs of the ciliary body cells in the rabbit eye. Figure 8A is the micrograph of ciliary body cells isolated immediately after sacrifice and Figure 8B is the thin section of similar cells incubated 24 h in 10% serum containing medium. These figures demonstrate that after 24 h of culture the ciliary body cells retain nearly normal morphology with some swelling of mitochondria and other cytoplasmic organelles. The secretory folds (solid arrows) remain intact and the exocytotic secretory vesicles (open arrows) are also seen.

DISCUSSION

The data presented here clearly demonstrate that the water extractable IC factor depresses protein synthesis activities of the epithelial cells of the organ cultured intact lens. Furthermore, it was observed that the inhibitory effect is not uniform and lower molecular weight proteins showed a greater rate of diminution of their synthesis. Bands 8 and 9 of OCLE cells, which are mainly crystallins [Bagchi et al., 1987b], displayed near complete inhibition of their synthesis, whereas band 3 showed minimal reduction in its synthetic activity. The mechanisms involved in these specific inhibitory effects of IC are not yet clear. It is possible that the proteins

Fig. 5. Immunofluorescence pictures of lens cells in tissue culture with the control panel on the left (**A,C,E,G**) and the total IC treated panel on the right (**B,D,F,H**). A and B are phase contrast micrographs. C and D are anti-vimentin stained, and E,F and G,H are anti–α-tubulin and –α-actin stained, respectively. These micrographs clearly show that the presence of IC factor in the culture medium causes cell elongation. H shows higher amount of α-actin and abundant stress fibers. The magnification bars are 50 μm.

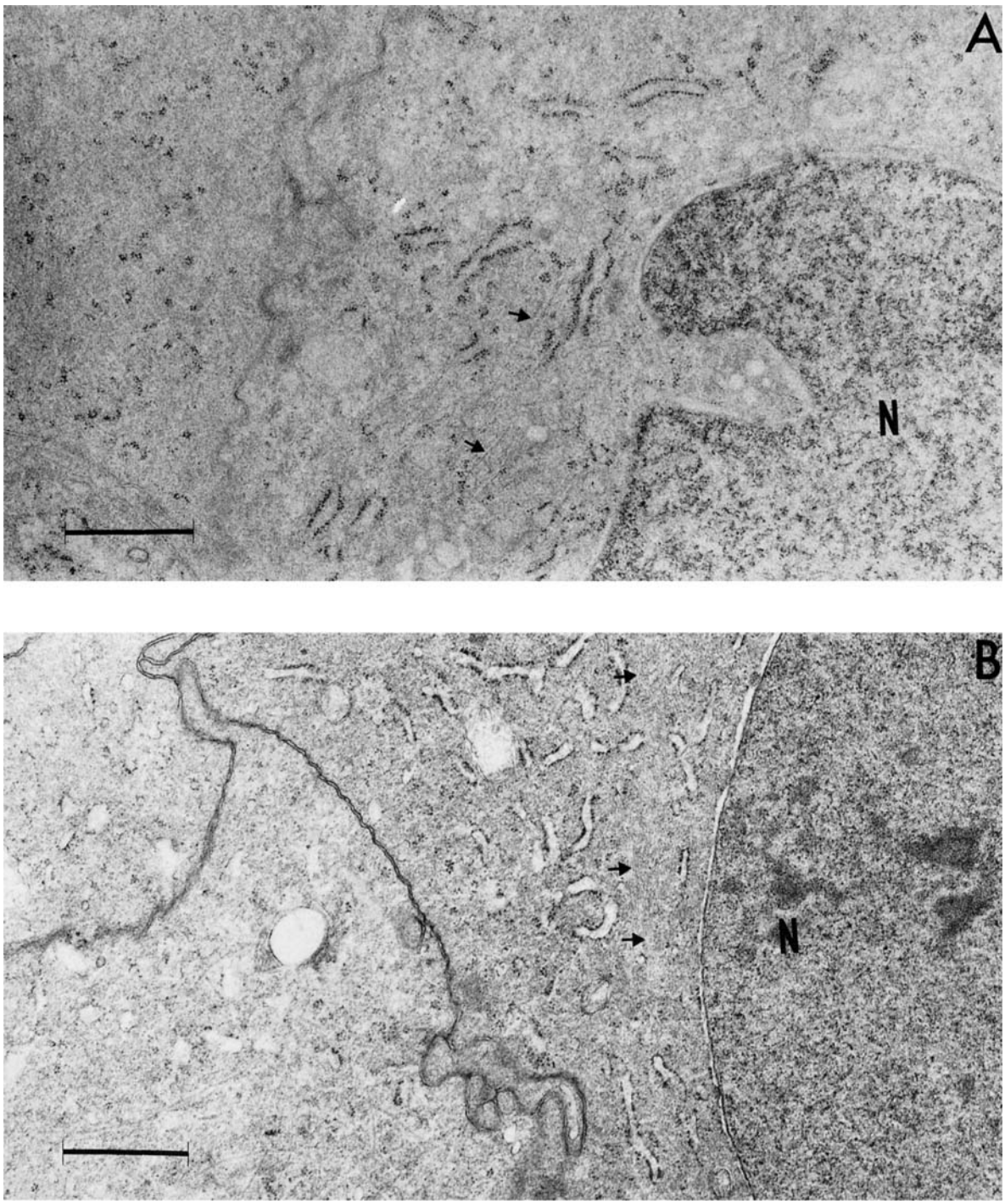

Fig. 6. Electron micrographs of thin sections through organ cultured lens epithelial cells. **A:** Normal control cell that was cultured with 10% serum for 24 h. **B:** Cell cultured for 48 h in medium 199. N, nucleus; arrows show the normal perinuclear distribution of the intermediate sized filaments. The magnification bar is 1 μm.

with slower turnover rate (see Table I) are more affected by IC than proteins with faster turnover rate. One of the most significant findings of this study is that the IC factors which inhibit protein synthesis by OCLE cells stimulate protein synthesis by TCLE cells. This observation suggests that the effect of IC on the epithelial cell protein synthesis is modulated by the organization of the intact lens. The intact lens is enclosed in a basement membrane-like capsule,

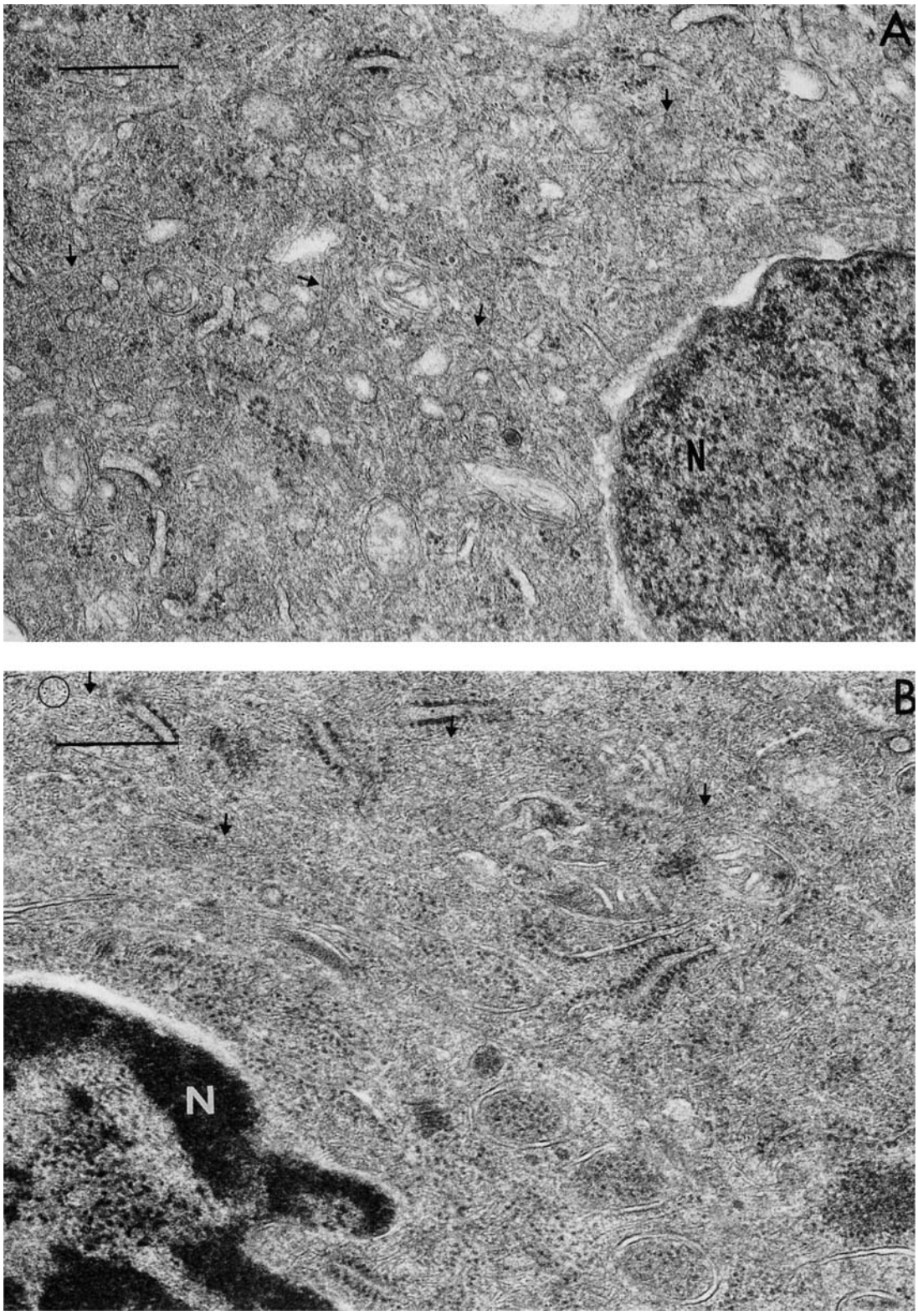

Fig. 7. Electron micrographs of thin sections of lens epithelial cells in organ culture with iris-ciliary body in the presence of 10% serum for 24 h, showing rearrangement of cytoskeletal intermediate sized filaments. The filaments are now dispersed throughout the cytoplasm (arrows). N, nucleus; the circle in **B** shows the filaments in cross-section. The magnification bar is 0.5 μm.

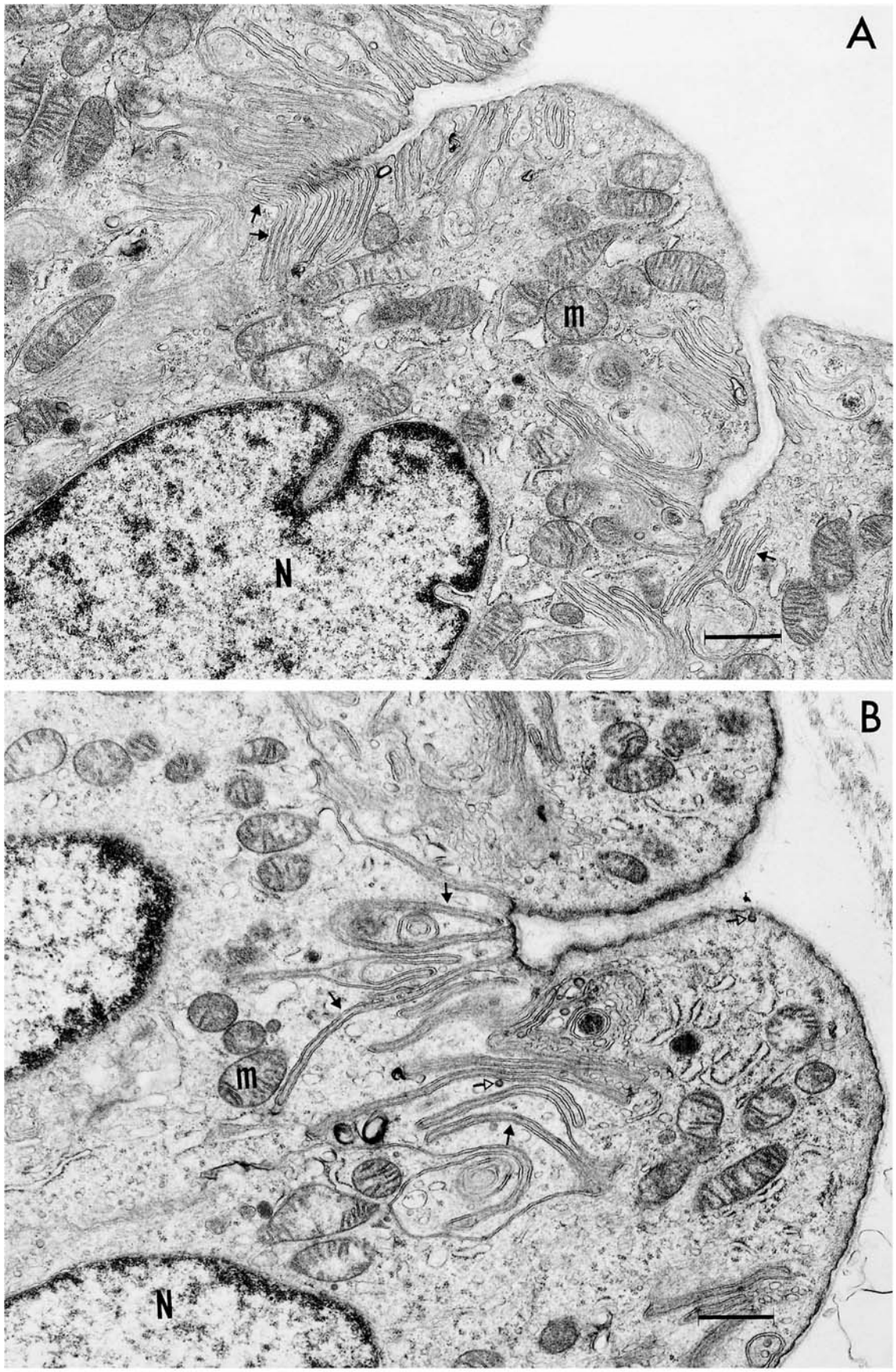

Figure 8.

and the monolayer epithelial cells are sandwiched between the capsule and differentiated fiber cells [Harding et al., 1971]. How this organization regulates the inhibitory effect of IC is not evident. However, basement membrane proteins like fibronection, entactin, laminin, etc., have been shown to function as external receptors for many growth regulatory proteins [Klagsburn, 1990]. It is also possible that the inhibitory effect of IC is modulated by cation levels of intact lens [Bagchi et al., 1987a; Piatigorsky, 1980].

It is evident from Figure 1 and Table I that the IC stimulates protein synthesis by TCLE cells. Both Figure 1 and Table I clearly demonstrate that all the polypeptides of the TCLE cells show marked enhancement in their synthesis rate. Some very low molecular weight proteins of TCLE, which are not detectable in OCLE, showed significantly increased synthetic activities. It is possible that these polypeptide bands represent breakdown product of higher molecular weight proteins. In summary, IC induces increased protein synthetic activities of TCLE cells and decreased synthesis of OCLE cell proteins.

The effect of IC on protein synthesis by OCLE and TCLE cells was also investigated using specific marker proteins. We selected three cytoskeleton filament proteins, vimentin, α-tubulin, and α-actin as marker proteins. We examined both synthesis and turnover rate of the marker proteins. An external agent which affects the turnover rate of cytoskeleton proteins can significantly alter homeostasis of the target cells [Otto et al., 1979; Franke et al., 1981, 1984; Henzen-Longmans et al., 1987; Bement and Capco, 1990]. The results from our experiments partially proved this hypothesis.

The marker proteins were visualized by immunoblot technique, thus assuring their chemical identity. The immunoblots of marker proteins were digitized for quantitative analysis. The analysis of digitized results clearly showed that both OCLE and TCLE cells have significant amounts of vimentin and α-tubulin and the presence of IC in the culture medium did not alter the total amount of these proteins. It was also found that TCLE cells have more vimentin and α-tubulin in comparison to OCLE cells. Generally, tissue cultured cells manifest increased amounts of vimentin and α-tubulin [Ramaekers et al., 1980, 1982; Bagchi et al., 1985]. Actin was not detectable by our technique in either treated or control OCLE cells; however, IC treated TCLE cells displayed significant amounts of α-actin.

The structure and composition of the cytoskeleton is supposed to be responsible for the maintenance of cell shape and function by serving as a network to transduce configurational and positional information to the nucleus [Penman et al., 1981] and cytoskeleton reorganization could influence synthesis of cytoskeleton proteins [Barcellous-Hoff and Bissel, 1989]. The IC treated OCLE and TCLE cells showed cytoskeleton reorganization. However, ^{3}H-leucine incorporation data revealed that IC has minimal effect on the synthesis of vimentin and induces a slight decrease in the synthesis rate of α-actin and α-tubulin in OCLE cells. Vimentin synthesis is generally regulated by growth stimulators and thus it is not surprising to observe minimal or no effect of IC on vimentin synthesis by OCLE cells [Traub et al., 1983; Jarvinen, 1990; Tsuru et al., 1990].

However, TCLE cells showed significant increase in the rate of α-tubulin synthesis in response to IC [Da Silva and Juliani, 1988; Ducommun et al., 1990; Fernyhough and Ishii, 1987; Fernyhough et al., 1989; Wheatley et al., 1988]. The α-actin and vimentin synthesis of TCLE cells was not significantly altered by IC. The stability and turnover rate of cytoskeleton proteins are essential for the maintenance of cytoskeleton structure and organization [Antras et al., 1989; Henzen-Logmans et al., 1987; Svitkina and Kaverina, 1989]. It was found that the IC enhanced stability of both vimentin and tubulin of OCLE cells and decreased stability of same marker proteins in TCLE cells. Vimentin has been reported to be a substrate for protein kinase C and stable vimentin may be involved in the phosphorylation of cellular and nuclear membranes [Huang et al., 1988]. It has also been shown that the IC can alter membrane activities of the intact lens [Bagchi et al., 1988]. The breakdown rate of vimentin and α-tubulin of OCLE cells was not affected by IC, but was significantly increased in TCLE cells. These data indicate that the IC activity is probably modulated by extracellular matrix (ECM), like cap-

Fig. 8. Electron micrographs of thin sections of ciliary body cells. **A:** Cell from freshly killed rabbit. **B:** Cell after 24 h in organ culture with lens cells in presence of 10% serum, showing comparable morphology. N, nucleus; m, mitochondrion; solid arrows, secretory folds; open arrows, exocytotic vesicles. The magnification bar is 1 μm.

sule, and without ECM mediation IC stimulates protein synthesis by TCLE cells [Giese and Traub, 1988]. The α-actin of OCLE cells displayed extensive increase in its turnover rate in response to IC. Many growth regulators are known to interfere with actin synthesis and breakdown [Liau et al., 1990; Miyamoto and Wu, 1990; Peterson et al., 1989; Serpinskaya et al., 1990]. Many growth stimulatory agents are known to decrease actin synthesis, whereas antimitogenic compounds stimulate actin synthesis. The quiescent smooth muscle cells express maximal levels of α-actin [Blank et al., 1988; Blank and Owens, 1990; Clowes et al., 1988; Wang and Rubenstein, 1988]. It can be postulated that the IC factor is a growth inhibitor and like many growth inhibitors it activates actin synthesis [Felipo and Grisolia, 1987; Rebillard et al., 1987].

The distribution and organization of cytoskeleton filaments of OCLE cells treated with IC were examined using immunofluorescence techniques. The vimentin and α-tubulin patterns of treated OCLE cells manifested a collapse of the perinuclear cytoskeleton to a dispersed and diffused pattern [Wang et al., 1989; Ciesielski-Treska et al., 1988; Ramaekers et al., 1982]. The actin pattern and amount were greatly altered in the OCLE cells. Thus, the cytoarchitecture of OCLE cells was significantly altered by IC.

The TCLE cells incubated with IC depicted extensive elongation and the hexagonal epithelial cells became long and slender fiber-like cells [Ringens et al., 1982]. However, the vimentin and α-tubulin organization of TCLE cells did not display any alteration in response to IC. The TCLE cells treated with IC showed significant increase in the cytoplasmic α-actin filaments and they were present mostly as stretch fibers. The IC factor induces many cytoskeletal changes in the epithelial cells of the organ cultured lens similar to that seen in other known differentiating cells [Bement and Capco, 1990].

The fine structure analysis of OCLE cells clearly demonstrated that the IC causes noticeable rearrangement of cytoskeleton filaments. The normal perinuclear α-tubulin and vimentin organization was altered and seen throughout the cytoplasm in the IC treated OCLE cells [Ramaekers et al., 1982]. These micrographs also demonstrated that the IC did not induce any significant morphological changes in the epithelial cells.

Earlier, we had reported that the presence of intact IC in the culture medium causes extensive cellular damage to the organ cultured lens [Niyogi et al., 1991]. These studies used light microscopy. However, electron micrographs of OCLE cells cultured with attached IC showed that not all the cells are damaged by IC, and those which are not damaged have near normal morphology. These studies indicated that there may be specific receptors present during particular stages of the cell cycle, necessary for the IC induced damage to the OCLE cells.

The fine structure analysis of co-cultured intact IC showed that IC retains its normal morphology for at least 24 h of culture and maintains its secretory activity, as evidenced by exocytotic vesicles.

In conclusion it can be postulated that the IC factor activity is modulated by a still unknown intermediary, as the epithelial cells of the whole lens show decreased protein synthesis in response to the IC factor, whereas in a similar medium the tissue cultured lens epithelial cells display increased protein synthesis. Is there any significant role these inhibitors of protein synthesis play in normal ocular physiology? It is possible that these factors are responsible for maintaining the central epithelial cells of intact lens in mitotic quiescence by inhibiting G_1-S phase transition [Weinberg, 1991; Banerjee et al., 1992]. Many growth suppressors induce differentiation [Weinberg, 1991], and the IC factor(s) could also be involved in the differentiation of the germinative zone epithelial cells of the intact lenses [Harding et al., 1971].

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