Influence of a prolonged period of low-dosage x-rays on the optic and ultrastructural appearances of cataract of the human lens

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SUMMARY Human lenses extracted for cataract 26 years after long-term exposure to an imperfectly shielded radium source were examined by slit-lamp photography, thin-section light microscopy, and electron microscopy. Anterior epithelial cells were fibroblast-like, and germinal epithelium and vacuolated cortical fibres had accumulated at the equator. A zone of light scatter at the anterior pole corresponded to an area of breakdown of cortical lens fibres, where unusual feathery fibres were orientated perpendicular to the lens surface. Two zones of light scatter separated by a 250-µm clear interval were seen in the posterior cortex. The zone at the posterior pole corresponded to an area of fibre liquefaction and large rounded membrane whorls, while the deeper zone comprised small flattened membrane whorls. The characteristic plaques of swollen abnormal cells described in previous histological studies of x-ray cataract were not present. This and other differences probably reflect the extremely long time course and repeated subliminal doses to which the patient was exposed.

Previous histological studies of both human and experimental x-radiation cataracts have mainly dealt with short-term changes developing after high radiation doses (for references see Duke-Elder, 1972; Hanna, 1975; Palva and Palkama, 1978). These studies indicate that x-rays act primarily on the germinal epithelial cells at the lens equator, and the cells divide to form defective lens fibres, which accumulate in plaques near the anterior and posterior poles. The breakdown of these defective fibres gives characteristic opacities in an anterior subcapsular zone and disc-shaped opacities in the posterior cortex (Hogan and Zimmerman, 1962).

In this study slit-lamp photography, and light and electron microscopy are used to investigate cataractous changes in human lenses which appeared many years after prolonged exposure to repeated low doses of x-radiation over a period of 11 years. The structure of the lenses is compared with that found in previous studies of x-ray cataract, in normal lenses (Hogan et al., 1971), and senile cataract.

Materials and methods

The lenses were obtained after routine cataract extraction from a patient whose visual acuity had been reduced to 6/24 in each eye. Before cataract

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extraction the patient had been subjected to an imperfectly shielded radium source (120 mg) for a few minutes 3 times a week for a period of 11 years (1935–50). From 1950 there had been no exposure to radiation until removal of his cataract 26 years later.

Whole lenses were fixed by immersion in 4 changes of 3% glutaraldehyde in 0·1 M Sorenson's phosphate buffer pH 7·3 containing 0·1% $CaCl_2$ for 4 hours at 4°C. They were washed 10 times with the same buffer with 9% sucrose added and postfixed in 4 changes of 1% OsO_4 in White's solution (Wakely, 1974) overnight at 4°C. The lenses were bisected through the lens axis, block stained with buffered uranyl acetate (Brightman and Reese, 1969), dehydrated, and then embedded in Araldite. Light and electron microscope sections were cut approximately perpendicular to the surface of the lens in the regions of the anterior pole, equator, and posterior pole. Light microscope sections were stained with 1% toluidine blue/borax and electron microscope sections with ethanolic uranyl acetate and alkaline lead citrate (Reynolds, 1963).

Results

SLIT-LAMP APPEARANCES
Fig. 1. View of anterior cortex. There is a dense area of scatter just below the capsule in the region of the anterior pole of the lens, and also isolated areas
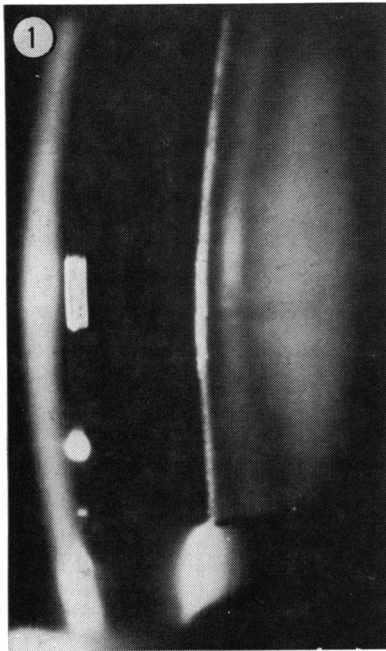
Fig. 1 *Slit-lamp view of anterior portion of the lens showing subcapsular opacification*

Fig. 2 *Slit-lamp view of posterior portion of the lens showing two areas of scatter separated by a clear interval*
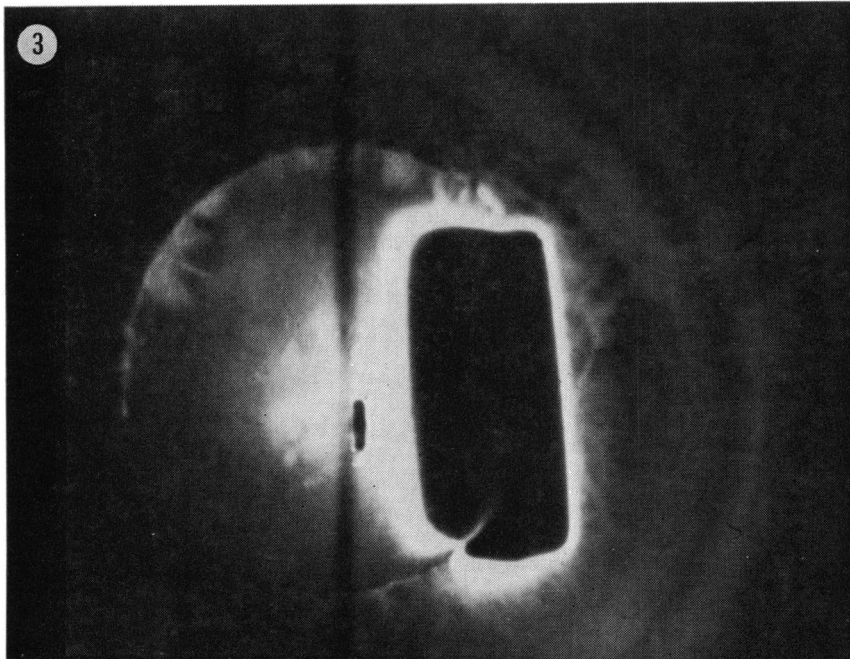

Fig. 3 *Slit-lamp view of posterior portion of the lens by reflected light showing some isolated areas of scatter at the periphery of the opacity*

which are again subcapsular out as far as the edge of the iris. The electron microscopy of these areas is shown in Figs. 4–10 and corresponds to feather fibres and the large vacuolated cells.

Fig. 2. View of posterior cortex. Two yellowish areas of scatter are seen in the posterior cortex separated by a clear interval. At the posterior pole of the lens the scatter area corresponds to Fig. 11. The other area between the posterior pole and nucleus corresponds to Fig. 14. Neither area has a

Fig. 4 *1 μm light microscope section of the anterior capsule, epithelium, and lens cortex. The asterisk marks a local thickening of the lens capsule from 15 to 28 μm. The spindle-shaped epithelial cells (e) are very darkly stained. Lens fibres show swelling, shrinkage, and dark granules. ×490; scale line 10 μm*
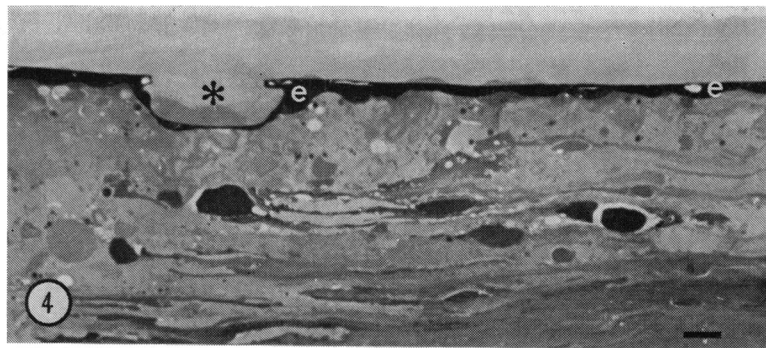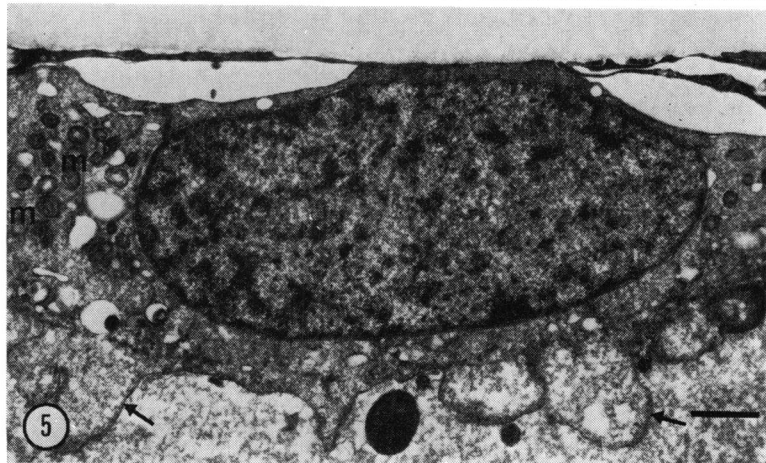

Fig. 5 *Spindle-shaped anterior epithelial cell with rounded processes (arrows) extending into the cortex. The cell contains many densely stained mitochondria (m). ×8900; scale line 1 μm*

typical bivalve appearance which is seen in severe radiation cataract, for example, atomic bomb radiation. In addition at the periphery there are some irregular areas of scatter which do not correspond to cuneiform-like opacities which would be seen on retro-illumination. These areas produce scatter by reason of the irregularity of the lens fibres, which are overlaid by vacuolated areas (Fig. 10).

Fig. 3. View by retro-illumination. A circular area of increased scatter is shown at the posterior pole of the lens together with some irregular areas at the lens periphery. These appearances do not show the markedly increased scatter at its edge producing a ring-shaped bounded area characteristic of acute radiation cataract. However, the margins of the area are more clearly defined when compared with senile cupuliform cataract. (From the measurement of slit-lamp photographs the clear interval between these areas is about 250 μm thick. This corresponds to a period of about 30 to 35 years for the fibres to be laid down [Fisher, 1970].)

LIGHT AND ELECTRON MICROSCOPY
Anterior lens. The thin epithelium at the anterior

pole (1 to 3 μm thick) comprised darkly stained spindle-shaped cells up to 30 μm long (Fig. 4). Occasional breaks were found in the epithelium, and many cells contained large vacuoles. The capsule at the anterior pole was usually about 17 μm thick, but a small area was found where capsule thickness increased to 29 μm (Fig. 4); the spindle-shaped epithelial cells were here depressed into the lens cortex. Away from the anterior pole the epithelium was thicker (5 μm) and the cells were less densely stained. These epithelial cells were unusual in having many dark mitochondria and a number of rounded projections into the lens cortex (Fig. 5).

An area of liquefaction of the superficial cortical lens fibres, 200 μm deep, was observed at the anterior pole, containing feathery filaments orientated mainly perpendicular to the lens surface (Fig. 6). Electron microscopy showed that the matrix of this watery area and of the filaments resembled the fine granular cytoplasmic matrix of intact lens fibres (Fig. 7). The feathery filaments, a maximum of 20 μm wide and 200 μm long, were only partly contained by cell membrane, junctional membranes being particularly well preserved. Among the filaments dense granules
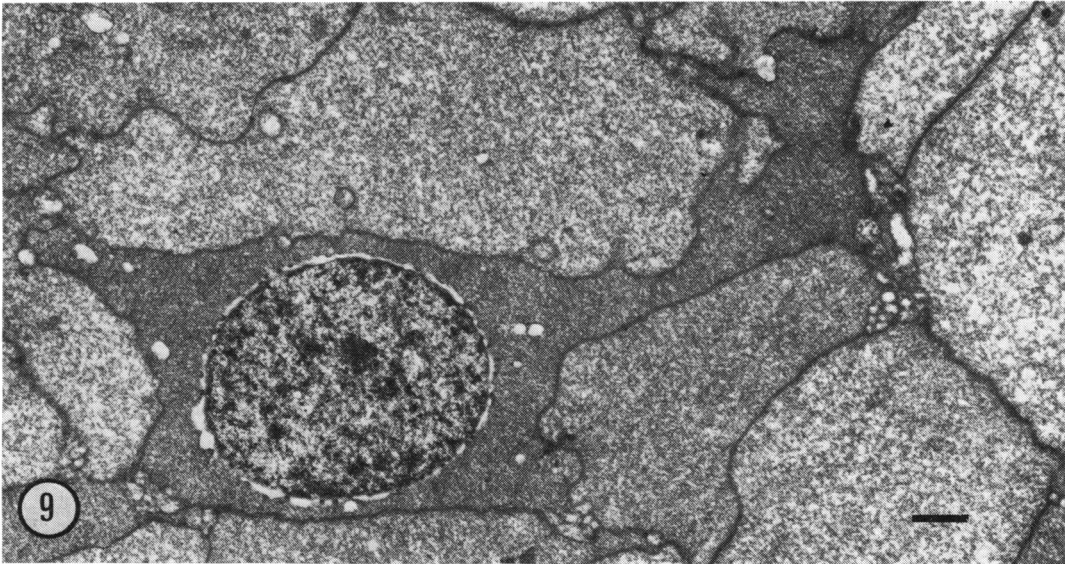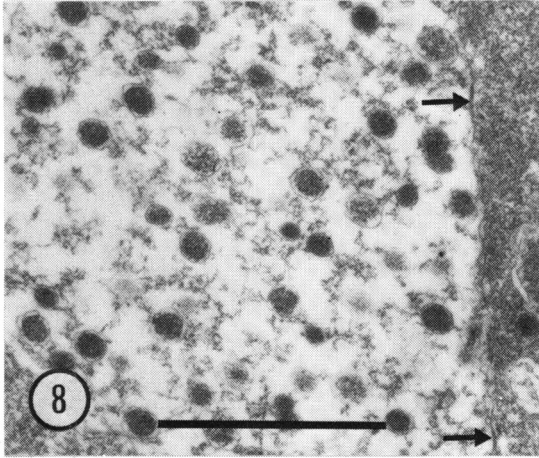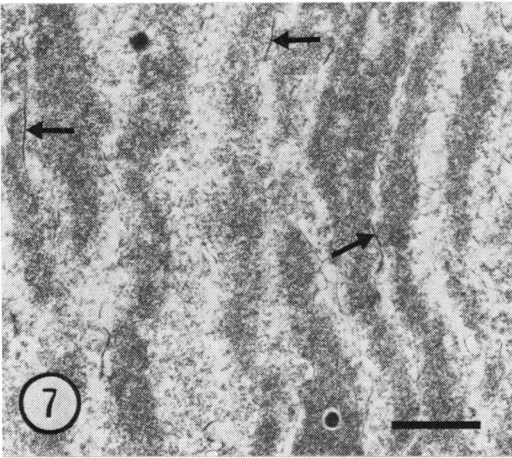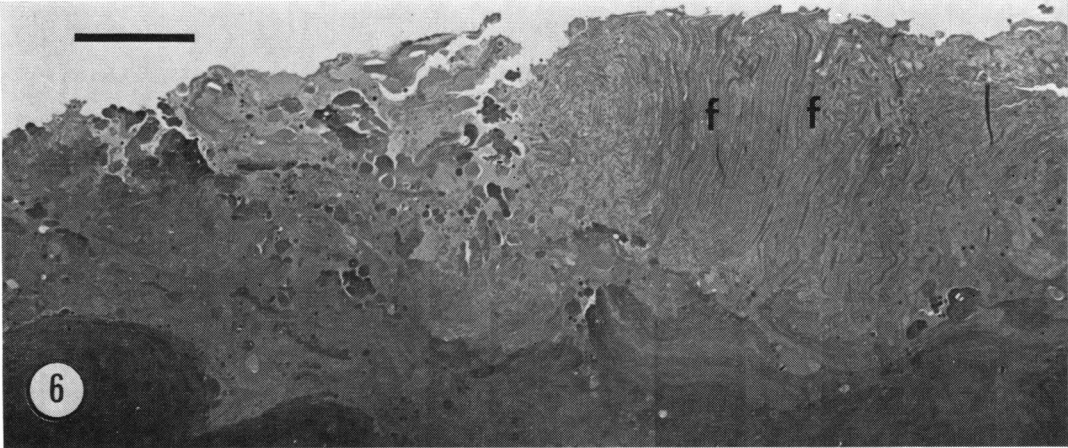
(0·1 to 0·3 μm diameter) were found. At the edge of the filamentous area cortical lens fibres were swollen and dense membrane-bound granules, up to 1 μm diameter, were common (Fig. 8). Swollen lens fibres reached 10 μm diameter and were bounded by fragmentary cell membranes, occasionally fused to small membrane whorls (Fig. 6). Other superficial cortical fibres (to a depth of about 50 μm) had a more normal ultrastructural appearance although they varied in diameter (up to 8 μm) and were undulating in outline (Fig. 9). Nucleated fibres with amoeboid outlines were occasionally seen among them (Fig. 9). Deeper cortical fibres were dense and shrunken with wavy outlines (Figs. 4 and 6).

Equator. Towards the equator the epithelial cells gradually became less spindle shaped and more cuboidal in shape, the epithelium was about 10 μm thick (normal thickness 15 μm), and the cells contained many vacuoles next to the capsule (Fig. 10). Between the epithelium and lens fibres was a darkly stained layer about 0·5 μm deep. At the equator the germinal epithelium formed a plaque of cells with oval palely stained cell bodies about 3 cells (22 μm) thick. Extending anteriorly beneath the epithelium and posteriorly beneath the capsule for about 200 μm was a layer of unusually fine fibres with diameters of 1 μm or less and containing many small vacuoles. Beneath these fibres the superficial cortex appeared to be normal to a depth of 90 μm where fibre swelling was marked. Deeper in the cortex fibres were darkly stained, undulating, and fragmented as in the deep anterior cortex.

Posterior lens. The lens capsule at the posterior pole, which had artefactually lifted away from the cortex, was of normal thickness (approx. 3 μm). The posterior pole showed liquefaction of cortical lens fibres to a depth of 150 μm in the right and 500 μm in the left lens. The watery area contained little granular or filamentous debris when compared with the anterior pole (compare Figs. 6 and 11). Debris in the watery area was in the form of dense membrane-bound granules up to 5 μm diameter and membrane fragments and whorls (Fig. 12). At the margin of the area of liquefaction the shrunken wavy cortical fibres had swollen rounded tips (Fig. 11), and similar rounded profiles with a matrix like that of cortical lens fibres were found in the watery area (Figs. 11 and 12). Subcapsular fibres at the posterior surface to the side of the watery area were disorganised to a depth of 40 μm, some being swollen while others were dense and appeared shrunken (Fig. 13). Large rounded membrane whorls, maximum diameter 2 μm, were found between the fibres, made up of stacks of fused membranes with a complex folded pattern (Fig. 13). In the right lens a further zone of membrane whorls was observed parallel to the posterior lens surface and localised beneath the watery area at 200 to 260 μm depth. Here the membrane whorls were flattened parallel to the lens fibres and the fused membranes continuous with the lens fibre cell membranes (Fig. 14).

Discussion

When the fine structure of the cataractous lenses is compared to that of the normal human lens (Hogan *et al.,* 1971; Kobayashi and Suzuki, 1975), the greatest changes are seen in the lens epithelium, anterior and posterior cortex. The lens capsule has a normal appearance and thickness except for a local thickening near the anterior pole, where the epithelium is depressed into the cortex. Local depressions of this type are thought to be the sites of elimination of lens fibre debris into the capsule and are common in senile cataract (Brini *et al.,* 1963). In a histological study of ionising radiation cataracts by Cogan *et al.* (1952) thickening of the posterior capsule was, however, consistently observed.

Other common features of previous histological studies of *x*-radiation cataract are subcapsular plaques of epithelial cells, particularly at the posterior pole, where these are often swollen to form bladder or Wedl cells (Cogan and Donaldson, 1951; Cogan *et al.,* 1952; Fujinaga, 1973; Hanna, 1975; Palva and Palkama, 1978). No plaques are found in this study, but rare nucleated lens fibres occur in the anterior cortex. The anterior epithelium is extremely thin

Fig. 6   *Light micrograph of lens cortex near the anterior pole. Many swollen and shrunken lens fibres and fine granules can be seen on the left of the micrograph, and on the right feathery fibres (f) are orientated perpendicular to the lens surface. The capsule was lifted away from the anterior lens during dissection.* × *160; scale line 100 μm*

Fig. 7   *Electron micrograph of the feathery fibres shown in Fig. 6. Fragments of cell membrane occur between them. Junctional membrane: arrows.* × *11 800; scale line 1 μm*

Fig. 8   *Intracellular granules and junctional membranes (arrows) of the swollen lens fibres in the anterior cortex. The dense granules have a similar matrix to the lens fibres and most are approximately 0·1 μm diameter.* × *30 000; scale line 1 μm*

Fig. 9   *Nucleated cell in the anterior cortex has processes extending between the swollen misshapen lens fibres.* × *7600; scale line 1 μm*
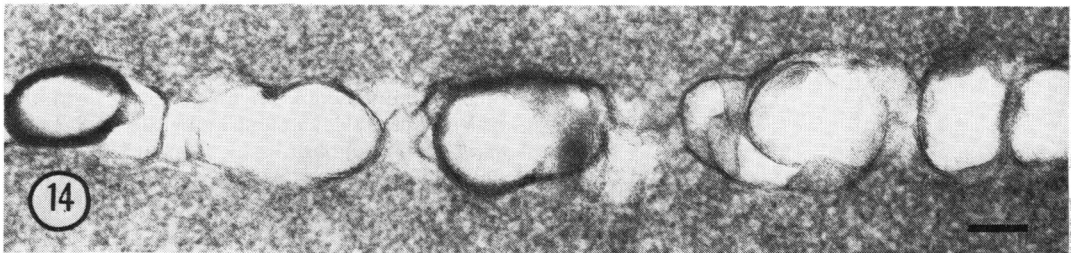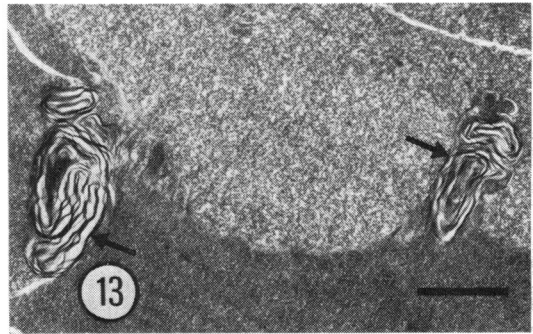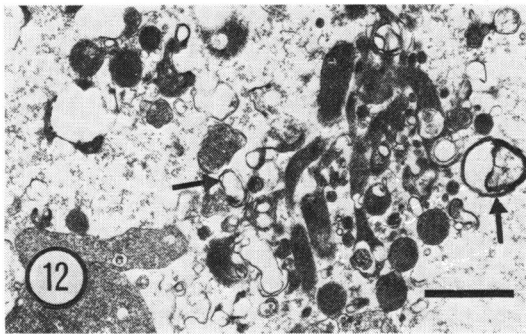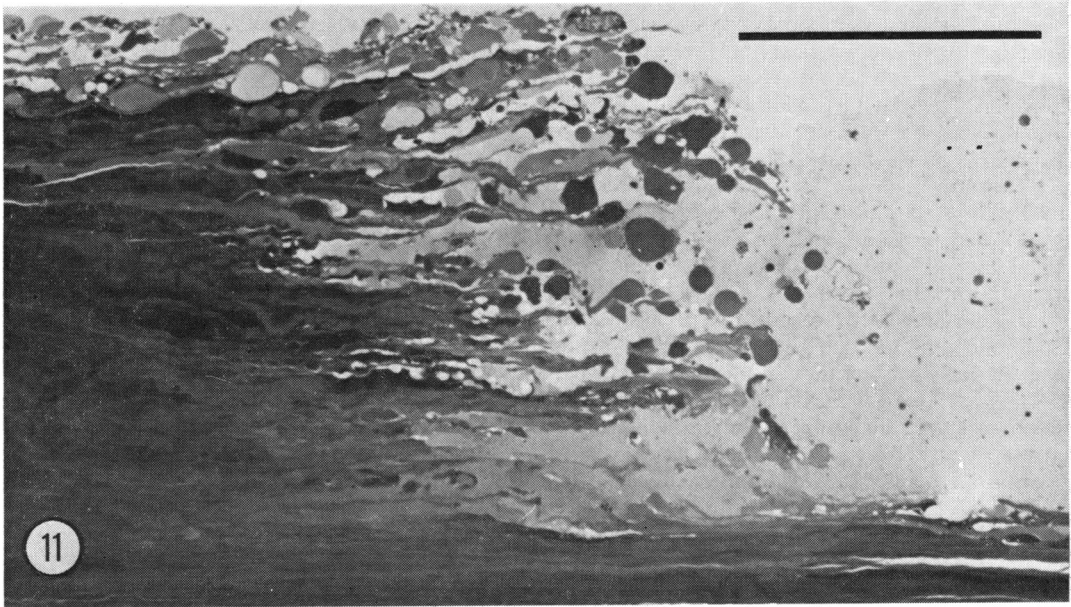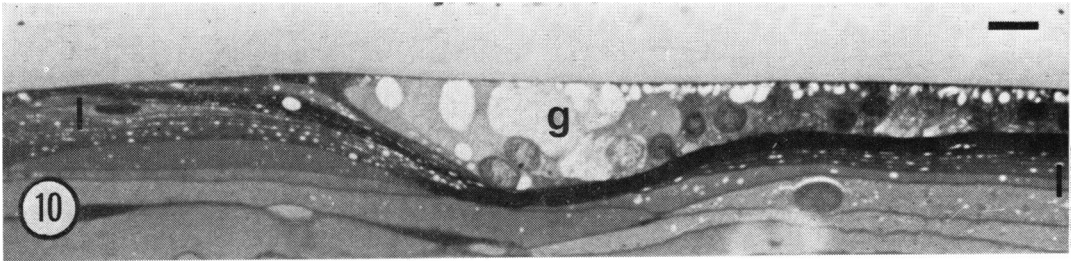
and discontinuous; the epithelial cells are spindle shaped in section and about 30 μm diameter instead of their normal 20 μm (Hogan *et al.*, 1971). There is reduced interdigitation between the cells; they contain many darkly stained mitochondria and have projections into the cortex (see Palva and Palkama, 1978). This patchy, fibroblast-like anterior epithelium has previously been found after irradiation (Cogan *et al.*, 1952; Hanna, 1975).

Low doses of radiation are thought to act on the germinal epithelial cells at the lens equator causing accelerated defective cell division (Hanna, 1975). Lens fibres formed from these cells fail to elongate, lose their cellular attachments, and become vacuolated. In this study an accumulation of epithelial cells and shrunken vacuolated cortical fibres occurs at the equator suggesting that the germinal epithelium has been damaged and is unable to form normal lens fibres. Other superficial equatorial cortical fibres are slightly swollen but otherwise normal (Hogan *et al.*, 1971). Deeper cortical fibres are darkly stained, shrunken, and fragmented like normal nuclear fibres or the cortical fibres of senile cataracts (Kobayashi and Suzuki, 1975).

Zones of cortical liquefaction are found at the anterior and posterior poles consistent with the clinical appearance of late stage *x*-ray cataract, that is, disc-shaped polar cortical opacities (Duke-Elder, 1972). At the edges of the liquefied zones a

Fig. 10 *Lens equator. Epithelial cells and superficial cortical lens fibres are vacuolated. Germinal epithelial cells (g) have accumulated at the equator and a layer of thin vacuolated lens fibres (l) can be seen in the superficial cortex. ×650; scale line 100 μm*

Fig. 11 *Posterior cortex. Shrunken wavy lens fibres on the left of the micrograph have swollen tips extending into the area of liquefied lens cortex on the right. Round granules are suspended in the liquefied area which is near the posterior pole. ×400; scale line 100 μm*

Fig. 12 *Debris in the liquefied area of the posterior lens cortex consists of membrane-bound granules and membrane whorls (arrows). ×11 800; scale line 1 μm*

Fig. 13 *Posterior cortex bordering the liquefied area. Large rounded membrane whorls (arrows) are found between the distorted superficial cortical lens fibres just below the capsule. ×11 800; scale line 1 μm*

Fig. 14 *Flattened membrane whorl in the posterior cortex at a depth of about 250 μm below the capsule. Up to 5 membranes are fused together to form the whorl. ×78 000; scale line 0·1 μm*

variety of degenerative changes are seen in the cortical lens fibres—shrinkage of fibres and breakdown of their cell membranes, swelling of fibre tips, and the formation of small dense intracellular membrane-bound granules. The watery areas contain the products of this degeneration. For instance, the large round globules are probably the pinched-off swollen tips of cortical fibres and may correspond to the morgagnian globules found in more mature cortical cataracts (Hogan and Zimmerman, 1962). Membrane fragments and small dense granules are also found here.

The most striking feature of the anterior area of liquefaction is the feathery fibres orientated perpendicular to the lens surface, bounded by incomplete cell membranes. These shrunken realigned cortical fibres do not appear to have been described in previous histological studies of cataract. In the posterior cortex bordering the watery area damage to the cortical fibre cell membranes is apparent both as fragmentation and as large rounded membrane whorls of fused cell membranes. Somewhat larger whorls are found in mature posterior cortical cataract (Hayes, unpublished). The yellowish area of light scatter seen in the slit lamp at the posterior pole probably includes both the watery area and the surrounding region of cortical fibre membrane damage.

A deeper zone of light scatter between the pole and the nucleus corresponds closely in depth and lateral spread with the distribution of flattened membrane whorls. This may be the original zone of radiation damage to the posterior lens, subsequently buried in the lens cortex by the growth of more normal lens fibres in the 26 years after exposure to radiation (see Fisher, 1970). Fisher and Wakely (1976) showed experimentally that zones of light scatter in the lens can be produced by membrane whorls which develop several years after damage to the capsule, and Philipson (1973) has also suggested that membrane whorls cause a loss of transparency in supranuclear cuneiform cataract.

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