Results. Bovine parathyroid catecholamines. Slices of bovine parathyroid tissue contained large amounts of dopamine (3.4–13.9 pg/μg). Norepinephrine values were insignificant (less than twice blank). The detection limit for norepinephrine in this assay is 5 pg.

Histofluorescence studies. Only rare green fluorescent norepinephrine-containing nerve endings were observed terminating on parathyroid vasculature. No definite nerve endings were identified on endocrine parathyroid cells per se, which exhibited reddish autofluorescence. Large numbers of single, yellow-green fluorescent cells were observed, scattered diffusely throughout the connective tissue stroma of the gland (figure 1). At higher power, these fluorescent cells could be seen containing greenish-yellow granules, which in some cases appeared to have been released from the cells (figure 2).

Discussion. Unlike the human parathyroid gland[12], we found no evidence of direct catecholamine innervation of parathyroid cells in the bovine parathyroid gland. Sparse norepinephrine-containing nerve endings were observed, but these appeared to terminate exclusively on small blood vessels. This confirms previous electron microscopic evidence of vascular innervation in the other species[17]. Thus, if the α-[5] and β-adrenergic[2-4] receptors on the bovine parathyroid cell are of physiologic importance, they must mediate responses to circulating epinephrine. There is, at present, no evidence in this species for a role of circulating catecholamines in modulating parathyroid function in vivo. The presence of large quantities of dopamine in the bovine parathyroid gland is of some interest. By histofluorescent microscopy, dopamine appeared to be localized to single cells scattered diffusely throughout the bovine gland in the connective tissue septa. The bovine parathyroid has been shown previously to contain an abundant number of mast cells[18]. Moreover, bovine mast cells contain large amounts of dopamine[19]. It is likely, therefore, that dopamine-containing cells within the parathyroid represent mast cells. We have demonstrated that dopamine causes 30- to 40-fold increases in intracellular cAMP in dispersed bovine parathyroid cells with concomitant 2- and 4-fold increases in parathyroid hormone release[6]. It is conceivable, therefore, that release of dopamine from bovine parathyroid mast cells might indirectly stimulate parathyroid hormone secretion. Although various stimuli regulate mast cell release (e.g., immunologic), adrenergic and cholinergic[20,21] stimuli may modulate the release reaction. A more direct experimental approach will be required to determine if an analogous mechanism is involved in the regulation of parathyroid function.

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Hypophysectomy exerts a radioprotective effect on frog lens[1]

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Summary. Exposure to X-rays usually causes cataracts in frogs. These cataracts are always preceded by misalignment of the structures called meridional rows (MR). When cell division is completely halted by hypophysectomy, however, irradiation no longer disturbs the orientation of the MR. Since the MR are the structures formed as lens epithelial cells differentiate into lens fibres it is reasonable to propose that radiocataractogenesis depends upon a mitosis-driven formation of pathological fibres from epithelial cells that have been rendered abnormal by exposure to X-rays.

Though he did not choose this interpretation himself, the early work of Chalupecky[3,4] raised the possibility that X-rays cause cataracts. The older literature is reviewed in Poppe's dissertation[5]. Worgul and Rothstein recently reemphasized the suggestion that the formation of radiation cataracts depends upon injury to the lens epithelium that eventuates in pathological fibre formation[6]. A strong element in their working hypothesis is that cell proliferation is required in order to transform the damage sustained by the lens epithelium into abnormal fibres[7].

Figure 1 shows the important cytoarchitectural features of the frog *(Rana pipiens)* lens. It was noted by Worgul and Rothstein that whenever cataracts developed, the region known as the meridional rows (MR) was disorganized. Where the mitotic index was found to be naturally low (as in a population of animals from South Dakota) or where it

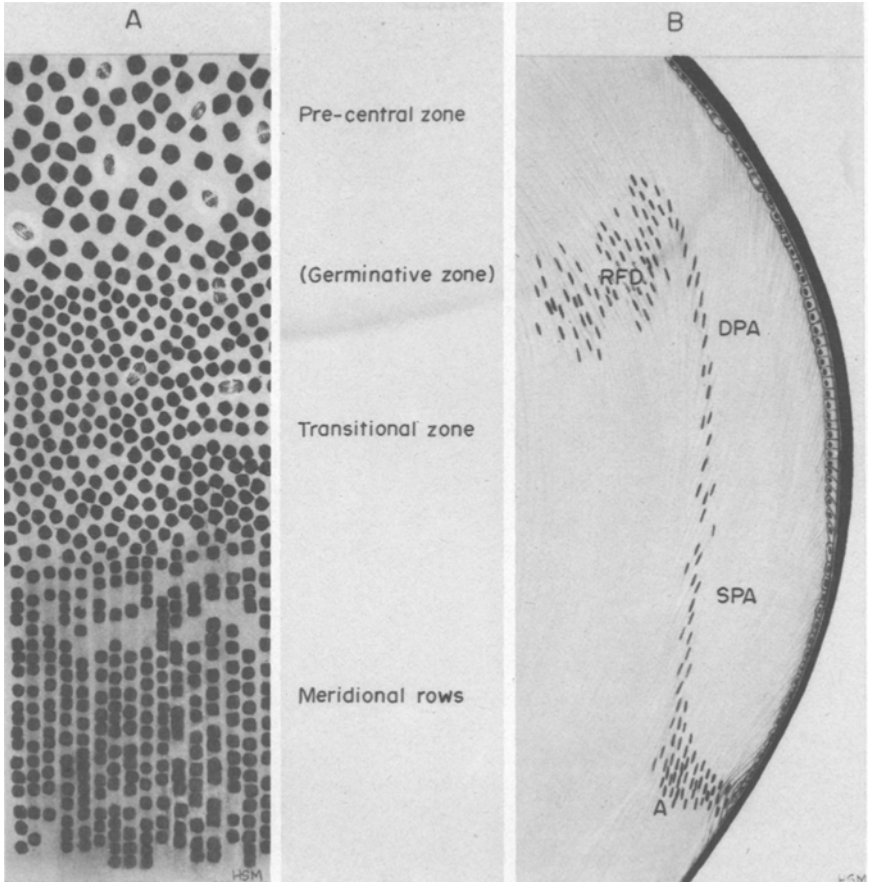

Fig. 1. This drawing shows a portion of a whole mount (A) and a section (B) of the lens. Mitosis is confined to the germinative zone. Upon completion of cell division cells migrate through the transitional zone and line up into meridional rows. Here the lens epithelial cells differentiate into lens fibres that are continually pushed deeper into the organ. During differentiation the nuclei migrate inside the lens fibre in a characteristic manner. They form the arc of the lens bow (A); superficial post arcuate zone (SPA), deep post arcuate zone (DPA) and region of fibre denucleation (RFD).

had been markedly lessened (low temperature treatment), cataracts did not form readily and the MR were not found to be in disarray.

These findings suggested a relationship between the MR and mitosis. Hayden and Rothstein also observed a probable correlation between mitosis and fibre formation[8]. In hypophysectomized *Rana pipiens,* mitosis stops entirely, 3 weeks after surgery. In these frogs no further migration of cells from the germinative zone to the bow of the lens (figure 1) occurs and fibre formation seems to come to a halt. (The state of mitotic arrest can be reversed by administration of growth hormone, frog prolactin, TSH, T_3 or somatomedin-C)[9–12].

The hypophysectomized frog provides us with a lens in which morphogenesis (i.e., the fashioning of fibres from epithelial cells) has been brought to a standstill. Our hypothesis of radiocataractogenesis would predict that the lens of a hypophysectomized animal should be insensitive to some of the effects of X-rays. The data to be presented bear out this prediction.

Materials and methods. The animals used were 6 cm *Rana pipiens pipiens* obtained from the lake Champlain frog farm, Alburg, Vermont. Animals were maintained in environmental chambers at a temperature of about 24 °C. 2 groups of animals were studied: hypophysectomized frogs and intact frogs. Hypophysectomies were performed by the procedure of Hogben[13]. 1 week after hypophysectomy these frogs received an injection of ^{3}H-thymidine via the dorsal lymph sac, at a dose of 1 µCi/g b.wt (sp. act. 6.0 Ci/mM). The intact frogs were injected with ^{3}H-thymidine 1 day before irradiation (1.5 µCi/g b.wt). The right eye of the animals was placed under the collimator of an X-ray source.

The collimator was fitted with a lucite guide; a dot on the tip of this guide served as a target to position the source directly above the eye at a fixed distance of 18.5 cm from the center of the pupil. The X-rays (185 kVp; 30 mA) were filtered by 0.5 mm Cu and 0.5 mm Al to provide a half value layer equal to 1 mm Cu; the exposure rate delivered to the plane of the target was measured with a Victoreen air chamber (Victoreen Instrument Corp., Cleveland, Ohio, USA) as well as a Baldwin-Farmer condenser chamber (Isotope Development Ltd, England), both of which were calibrated at the National Bureau of Standards. On the basis of these measurements, the dose rate was calculated to be 600 rad/min. The animals were irradiated 3–4 weeks after hypophysectomy, a time when all cell proliferation in the lens has ceased. Nonirradiated intact and hypophysectomized animals served as controls.

Slit lamp examinations were performed at various times after irradiation. Animals were sacrificed at several intervals up to 8.5 weeks postirradiation. After sacrifice the globes were removed and fixed in Carnoy's solution for 24 h, followed by 24 h in 70% ethanol. The lenses were removed and whole mounts of the epithelium were made by the method of Howard[14] as modified by Rothstein[15]. The whole mounts were prepared for autoradiography and stained with Harris hematoxylin.

Results and discussion. Figure 2a is a photomicrograph of a whole mount obtained from a normal, intact animal that was not irradiated. This animal received an injection of ^{3}H-thymidine 8 weeks earlier. The MR are in good alignment and labeled cells have migrated into them from the germinative zone by this time. Figure 2b shows a similar preparation from an animal that was irradiated and sacrificed 8.5 weeks later. Labeled cells have arrived in the MR and

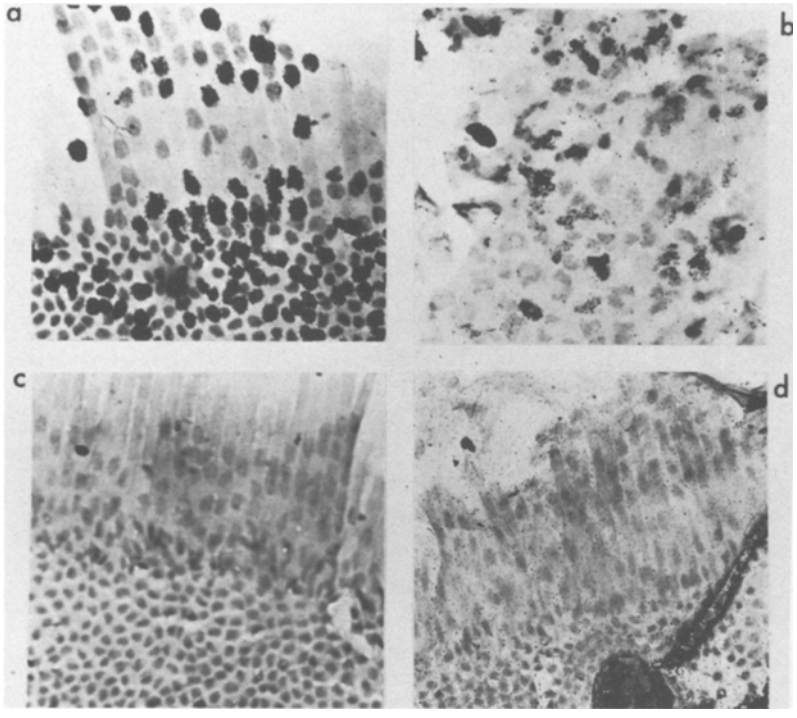

Fig. 2, a–d, *a* is an autoradiogram of a whole mount prepared from a normal intact animal 8 weeks after labeling with ^{3}H-thymidine. Labeled cells have entered the MR which are in good alignment; *b* shows a similar preparation from a animal that was irradiated and sacrificed 8.5 weeks later. Labeled cells have entered the region of the MR (which have become disorganized); *c* shows a whole mount from a non-irradiated, hypophysectomized frog. The animal received ^{3}H-thymidine 1 week after hypophysectomy and was sacrificed 82 days later. Note the ordered MR and the absence of labelled cells; *d* shows a whole mount from an irradiated, hypophysectomized animal. This frog was operated on 23–24 days prior to irradiation. Here, also, no labeled cells have entered the MR and no disorganization has taken place.

the rows are in disarray. In Figure 2c is shown a whole mount from a non-irradiated animal that had undergone hypophysectomy. The frog was operated on, injected with ^{3}H-thymidine 1 week later (though on the wane, DNA synthesis is still in evidence at this time) and sacrificed 89 days post-hypophysectomy. No labeled cells have reached the MR and there is no disorganization of the MR. Figure 2d shows a similar preparation from an animal that was irradiated 24 days posthypophysectomy and sacrificed 8.5 weeks later. No labeled cells are in the MR and the region is not disorganized. Hence, a pathological alteration, found in every case in which cataracts have previously been observed, does not become manifest in animals whose lens epithelium has ceased to proliferate. It is held that this happens because cataractogenesis is fundamentally a pathological morphogenesis, a morphogenesis, which like its normal counterpart, does not occur in the absence of cell division.

In the reported studies disorganization of the MR was characterized by the presence, within the rows, of cells that were located in the germinative zone at the time of irradiation. In hypophysectomized frogs irradiation does not lead to row disorganization and no labeled cells have reached the MR.

In the rat lens, Worgul et al.[19] have shown that cells labeled with ^{3}H-thymidine, while in the germinative zone, appear in the bow 1 week later. This interval is far shorter than the latent period for formation of radiation cataract.

Taken together these observations indicate that the initial lesion in radiation cataract is suffered by lens epithelial cells located in the germinative zone at the actual time of exposure. These cells subsequently migrate into the MR which become disorganized concomitant with (and perhaps as a result of) the arrival of the migrating cells. Later, cells may move to the posterior region of the lens. In this abnormal location they undergo pathological alterations that eventuate in posterior opacities[16–19]. It is of interest that Streeten and Eshaghian have observed disorganized MR associated with various human posterior subcapsular cataracts[20].

The frog lens is the first one in which it has been possible to perform direct studies on the relationship of cell proliferation to morphogenesis and radiocataractogenesis. Such is the case because both mitosis and cellular migration are eliminated in the lenses of hypophysectomized frogs.

Longer term experiments are planned. If the hypothesis proposed is accurate, X-ray induced opacification should not appear unless cell division is restored. This can be achieved with one of the growth promoting hormones mentioned earlier. The behavior of repair mechanisms during the arrest of DNA synthesis should also be open to study.

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