Vitamin D$_2$ (40,000 IU) per 100 g body weight was administered daily in male albino rats. The levels of calcium and inorganic phosphate were determined in serum and lens. Lens calcium was elevated in the treated rats by 40%. Invest Ophthalmol Vis Sci 27:447-448, 1986

There has been a great deal of interest in understanding the regulation of calcium levels of biological tissues. The regulation of calcium levels has been studied in muscle, nerve, endocrine gland, as well as in some epithelia, including intestine, liver, and red cells; however, little is known about its regulation in eye, where it has significant functions in ocular tissues. One such function of calcium is concerned with the visual disturbance in eye lens. Calcium induces the formation of protein aggregates in lens homogenates that may lead to lens opacification. The level of calcium in cataractous lenses has been correlated with the extent of lens opacification. This may possibly be due to cell adhesion and the permeability of the lens, which are influenced by calcium so that disorders in the metabolism of calcium might contribute to a change in the ocular tissue.

The most important biological regulators for calcium are vitamin D, calcitonin, and parathyroid hormone. Among these, vitamin D has attracted considerable attention because of its characteristic properties. Vitamin D$_2$ is hydroxylated first by the liver at carbon-25 position and then by the kidney at carbon-1 position. This results in the production of biologically active form known at 1,25-dihydroxy vitamin D$_3$. The presence of readily measurable levels of 1,25-dihydroxy vitamin D$_3$ has been reported in the serum of male, non-spawning female, and spawning female trout. Although vitamin D is stored in the liver in humans, its role in calcium homeostasis in eye lens is not known.

The purpose of present investigation was thus to ascertain whether the lens of male rats have the capability to respond to the hormonally active form of vitamin D$_2$ in terms of the elevation in the levels of calcium in the lens matrix.

**Materials and Methods.** Male albino rats weighing (80–100 g) were acclimatized to laboratory conditions for 1 wk. The rats were then divided into two groups (I and II). Group I rats were injected daily with 40,000 IU of Vitamin D$_2$/100 g body weight intramuscularly while group II rats were injected with the same volume of oil base. Rats of both groups were fed a diet containing legume gram soaked in water. After 2 wk, the animals were fasted overnight, anaesthetised, and blood samples were withdrawn by hearth puncture. The animals were then killed, and the lenses were dissected from the eye ball. Sera were separated from the blood of centrifugation at 3500 rpm for 10 min. Lenses were homogenized in 1.0 ml phosphate buffer (pH 7.4, 0.1 M), and equal volume TCA (10%) was added to precipitate protein. Supernatants were obtained by centrifugation at 5000 rpm for 10 min. The levels of calcium and inorganic phosphate were determined following the method as earlier described.

The investigations described in this study were performed in accordance with the ARVO Resolution on the Use of Animals in Research.

**Results.** Vitamin D$_2$ is prohormone, which is converted to its biologically active form 1,25-dihydroxy vitamin D$_3$ before it functions in the maintenance of adequate amounts of calcium and phosphorous at the calcification sites. This results in the elevation of plasma calcium and phosphorous concentrations, which is achieved by stimulation of intestinal calcium absorption, of intestinal phosphorous absorption, as well as mobilization of calcium and phosphorous from bone. The results summarized in Figure 1 suggest that such an effect has been observed in the case of serum calcium and phosphorous levels that increased by about 40% and 37% respectively.

The variation in serum phosphorous levels does not lead to severe symptoms as happens in the case of variations in serum calcium levels. Interestingly enough, it was observed that the level of total calcium in lens increases by about 40% (Fig. 2). This is a significant result since such an observation had not been made earlier. The increased level of calcium had been observed earlier in cataract formation, but the mechanism of its increase has not been known thus far.

**Discussion.** Vitamin D is generally accepted as being essential for life in higher animals. Along with two peptide hormones, parathyroid and calcitonin, vitamin D is responsible for establishment as well as the maintenance of calcium homeostasis. A new concept
of the mechanism of the action of fat-soluble vitamin D has recently emerged. According to this concept, vitamin D is more properly considered as a steroid hormone than a vitamin in the classical sense. Besides, vitamin D endocrine system is comprised of many more target cells and tissues in addition to the intestine, kidney, and bone. These include the beta cells of the pancreas, breast tissues, placenta, the pituitary gland, and cells of reticuloendothelial system. Thus the results of the increased level of calcium in lens suggest that this may occur due to two alternatives: either excess calcium from blood reaches aqueous humor through blood aqueous barrier from where it possibly enters lens tissue or the lens may itself act as a new target site for the action of vitamin D2.

Vitamin D2 thus generates the characteristic physiological response leading to alteration of membrane structure considered necessary for elevation of calcium and phosphate levels.

It is thus possible that the functional behavior of vitamin D2 in relation to the increase in the level of calcium in eye lens may be considered essential from the viewpoint of the physiological disordering of the lens system. It has earlier been suggested that severe hyper-, hypoparathyroidism, as well as chronic renal diseases can be well understood in terms of disrupted or accelerated vitamin D metabolites.

Key words: vitamin D2, calcium, inorganic phosphate

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