dissolved in slightly acidulated water, a few milligrams of scandium chloride ($ScCl_3$) added to the solution and precipitated by an excess of ammonia. The precipitate exhibited an activity of the same character and comparable in amount with that emitted by irradiated potassium chloride.

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Carotenoids and the Vitamin A Cycle in Vision

SINCE reporting the occurrence of vitamin A in the eye tissues of the frog and several mammals[1], I have examined the carotenoids of the frog's eye in detail.

The combined pigment and choroid layers of *R. esculenta* and *pipiens* (dry weight about 2·2 mgm.) contain about 4γ per eye of vitamin A. There is also about 1γ of another carotenoid in these tissues possessing the spectroscopic and solution properties of xanthophyll. These quantities are not altered appreciably by light or dark adaptation.

In the retinas of dark adapted animals, no xanthophyll and only a trace of vitamin A occurs. Instead, their chloroform extracts contain a third carotenoid with novel properties. I have named this substance retinene.

Retinene in chloroform solution possesses no absorption bands in the visible spectrum. It is faintly yellow, due to an ascending absorption from 500 mμ into the ultra-violet. The crude retinal extract shows a small absorption maximum at about 410 mμ and larger ones at 310 and 280 mμ. Retinene exhibits a strong blue colour with antimony trichloride, due to a sharp band at 655 mμ.

Though present in quantity in the extracts of dark adapted retinas, retinene has completely vanished from light adapted ones. In these it has been replaced by about 0·3γ per retina (dry weight about 3 mgm.) of newly formed vitamin A.

The process which generates the vitamin is easily demonstrated in the isolated retina. Dark adapted retinas 'bleach' instantly in intense light to a bright orange colour (visual yellow). When such bleached retinas are immediately extracted with chloroform, they yield the same quantity of retinene as do dark adapted tissues, and no vitamin A. If, however, they are left at room temperature, the orange colour fades and within an hour has vanished. Extracts of such colourless retinas contain about 0·8γ per retina of vitamin A, and no retinene. Partially faded retinas yield intermediate quantities of both substances.

The fading of visual yellow proceeds equally well in light or in darkness, though in the latter instance some visual purple is regenerated. At 0° C. the process is delayed indefinitely, even in brilliant sunlight. The photochemical conversion of visual purple to visual yellow thus is followed by a thermal decomposition of the latter substance to colourless products, among them vitamin A.

Isolated retinas which have been bleached and have completely faded contain much more vitamin A than retinas from light adapted animals. Some vitamin A is therefore lost in the visual process.

Retinene is no more than a useful artefact in this system. It does not occur in the retina as a free substance. Benzine and carbon disulphide, though they dissolve both compounds easily, extract the vitamin A from dark adapted retinas, but no retinene. Subsequent extraction with chloroform yields retinene in the usual quantities. Neither carbon disulphide nor benzine affects visual purple or visual yellow, whereas chloroform rapidly decolourises both. Clearly vitamin A is bound in visual purple and yellow to some colourless molecule, insoluble in fat solvents. Chloroform breaks this complex to yield retinene. The thermal fading of visual yellow in the bleached retina dissociates the complex in another manner, liberating the vitamin.

Visual purple is non-diffusible[2] and may be salted quantitatively from solution with half-saturated ammonium sulphate. Its sensitivity to warming[2] and to deproteinating agents of all sorts adds a protein character to these general colloidal properties. The visual pigment seems, therefore, to be a carotenoid protein like that recently found by Kuhn and Lederer in lobster shells[3]. The lobster pigment and visual purple are similar in many properties; both are insoluble in water and organic solvents, and both are fundamentally altered by warming, acids, alkalis, alcohol and acetone. It is probable, therefore, that visual purple is a conjugated protein, in which vitamin A is the prosthetic group.

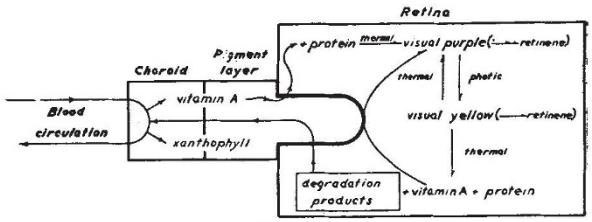

FIG. 1.

In isolated retinas which have been bleached and replaced in darkness, visual yellow reverts in part to visual purple; the remainder decomposes as described to colourless products. The latter substances never re-form visual purple in the isolated retina. In the intact eye this type of reversion does occur freely; it requires the co-operation of the pigment epithelium[2].

The pigment epithelium is necessary also as a source of vitamin A, quantities of which are lost during vision. These must be restored from the reserves in the pigment layer, since the frog retina contains no blood supply[4]. However, the ultimate source of vitamin A in any vertebrate is in the diet, and this is, at least in part, the reason for the failure of the visual purple mechanism (night blindness) in avitaminosis-A.

All these relations can be indicated in a single diagram (Fig. 1), which is tentatively proposed to represent the elements of visual purple-vision in the frog.

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