Hereditary primary pigmentary degeneration of the retina occurs in a number of experimental animals, the condition closely resembling retinitis pigmentosa in man (Cogan, 1950). In the rat, the mode of inheritance is as an autosomal recessive characteristic (Bourne et al., 1938), whereas in man the situation is more complicated in that at least seven different forms of inheritance have been recognized. In the affected rat and dog, retinal degeneration starts about 12 days after birth although true differentiation of the rod is not completed until about 4 weeks of age (Lucas, 1954).

Biochemical investigations

Material for biochemical investigations has been obtained mainly from the eyes of pink-eyed piebald agouti rats descended from the original strain of Bourne et al. (1938).

Carbohydrate and protein metabolism

The dependence of the retina, like the brain, on carbohydrate as a fuel for the supply of energy prompted early investigators to study retinal glucose metabolism in detail. Graymore et al. (1959) found decreases in the rate of anaerobic glycolysis but none of these changes could be related to visual cell degeneration. Reading & Sorsby (1962) investigated retinal aerobic glucose metabolism in detail using radio-isotopic methods. No striking differences in the overall pattern of glucose metabolism between normal and affected retinae were found during the age range 10–21 days after birth. Later, affected retinae showed decreases in lactic acid, carbon dioxide and amino acid production, together with decreases in rates of respiration and utilization of glucose. Affected retinae did not retain, intracellularly, amino acids formed from glucose, namely aspartate, glutamate and γ-aminobutyrate, whereas non-affected retinae did retain these amino acids. This change in permeability occurred in the affected retinae before the animals had reached the age of 21 days and suggested possible abnormalities in protein metabolism. In consequence, uptake, transport and incorporation of [14C]glycine into total retinal protein was studied in vitro and in vivo (Reading & Sorsby, 1964). Retinal tissue was incubated for 2h periods in vitro in a phosphate medium with added glucose and [U-14C]glycine. Retinae from litter mates of affected rats were compared with retinae from normal animals at 6–24 days after birth; the former showed a lowered rate of incorporation of label into protein as early as 6–8 days of age. No differences in uptake or transport of amino acid into retinal tissue were found between normal and affected retinae. The results obtained in vitro were confirmed by experiments in which incorporation in vivo was measured at intervals after subcutaneous injection of 10μCi of [14C]glycine into 8-day-old rats. Affected animals showed a slower turnover of retinal protein. The rates of incorporation of label into protein of the livers of both normal and affected animals were equal. The reduction in protein turnover together with the genetic nature of the condition suggested an anomaly in the production of an essential protein.

Metabolic pathways associated with vision

Metabolic systems associated with the visual cycle have received detailed investigation. For instance, the hexose monophosphate shunt was implicated in the normal functioning of the visual cycle by Futterman (1963). In the normal retina the activity of the hexose monophosphate shunt is low in dark adaptation with short bursts of activity when light falls on the retina owing to the metabolic 'coupling' between the enzymic (retinal alcohol dehydrogenase) reduction of all-trans retinal and oxidation of glucose by the dehydrogenases of the hexose monophosphate shunt through the common coenzyme NADP+.
Developing affected retinas, compared with normal retinas, showed a tendency towards an overall increase in the activity of the hexose monophosphate shunt (Reading, 1964). Bonavita (1965) produced confirmatory evidence by showing a significantly higher specific activity of glucose-6-phosphate dehydrogenase in affected retinas at 12 days of age (about 60% higher than normal).

Measurement of retinal alcohol dehydrogenase activity (Reading & Sorsby, 1966) showed that the enzyme increased in activity in normal retinas until the animals were one month old and then levelled off to reach a steady level of activity. In affected retinas the enzyme activity developed similarly until the rats were 2 weeks old, whereupon wide variations in activity were found. Subsequently, retinas from 4-week-old affected rats showed only 40% enzymic activity compared with the corresponding normal retinas; by this time the retina shows substantial degenerative changes.

Isoenzyme changes in retinal degeneration

Graymore (1964) separated five distinct fractions having lactic dehydrogenase activity from extracts of rat retina by electrophoresis on cellulose acetate. They consisted of two major discrete fractions, the 'M' or muscle and the 'H' or heart isoenzymes, the remaining three hybrids of these. The retina is characterized by having a predominantly 'M'-type pattern. Graymore (1964) found an anomalous isoenzyme pattern for lactate dehydrogenase in affected rat retinas, the 'M'-type isoenzyme being deficient from birth. He suggested that the 'M' fraction played an important role in the differentiation process in the retina.

Changes in ATPase

The implication of ATPase (adenosine triphosphatase) in the sodium pump in cell membranes and the changes reported by Dowling & Sidman (1962) in the electroretinogram at 22 days coinciding with degeneration of rod inner segments and nuclei in the affected rat, prompted Bonavita and his colleagues (Bonavita et al., 1966) to compare the development of activities of the ATPases in normal and affected rats. They found that Mg2+-activated ATPase and Mg2+-dependent Na+, K+-activated ATPase activities increased with postnatal development of the retina. However, they also found a pronounced decrease in Mg2+-activated ATPase activity in affected retinas.

Vitamin A metabolism

In a biochemical survey of retinitis pigmentosa patients in the Midlands area, ranging in age from 9 to 65 years and covering a period of 11 years, Campbell & Tonks (1962) concluded that the most significant finding was a persistently low concentration of vitamin A in the blood, although the range of values was substantially the same in normal and affected persons. Although the low amounts of vitamin A were found to persist in individual subjects over many years, they appeared to the same extent in affected and non-affected relations in the same family. They suggested that low vitamin A concentrations were a positive factor in the development of retinitis pigmentosa and that the vitamin might circulate in a form which the retina could not utilize. However, Arden in a subsequent investigation (G. B. Arden, personal communication) was unable to confirm the reduction in vitamin A blood levels in this condition. There are some indications that humans affected by retinitis pigmentosa may have a defective transport mechanism for vitamin A. Rahi (1972) reported a deficiency in the retinal-binding protein in the serum of patients.

Abnormalities in visual pigment metabolism

Dowling & Sidman (1962) in a comprehensive investigation reported an over-production of visual pigment in the eye of the rat affected by pigmentary retinal degeneration. An excess of rhodospin was found in a disorganized form lying between the pigment epithelium and the outer part of the rod cell layer. The rhodospin was in the form in which it appears in the outer rod sacs, i.e. as a double membrane lamellar structure. At 20 days of age, affected animals possessed twice as much rhodospin per eye as corresponding
normal animals and subsequently rhodopsin content in the affected eyes fell rapidly. They also showed that histological and electroretinogram changes could be delayed by keeping the affected animals in the dark from birth.

In an attempt to relate abnormalities in visual-pigment formation to visual-cycle function, Reading (1966) compared the distribution of retinal and retinol in normal and affected rat eyes under conditions of dark and light adaptation. In the normal retina, even after prolonged bleaching, the concentration of retinal was never less than 11–12 µg/g wet wt. of tissue. On the other hand, in affected animals bleaching produced a conspicuous depletion of retinal in the retina. In addition, dark adaptation in affected animals resulted in the regeneration of less visual pigment than normal. The most striking difference between normal and affected animals was seen in the retinol content of the pigment layers at 2, 3 and 4 weeks of age. In the eye of the affected rat concentrations of retinol after bleaching were at least twice those in the normal rat at corresponding ages, whereas wet weights of retina and pigment layers in normal and affected animals were remarkably similar.

Nature of the primary biochemical lesion

Consideration of the nature of the biochemical lesion must include the following observations: excessive local concentration of retinol in the pigment layers and indications of a rapid turnover of visual pigment in affected rats. However, two pieces of evidence for some time were irreconcilable; on the one hand, the early observation of the decrease in the rate of protein synthesis in affected retina (Reading & Sorsby, 1964) and on the other, Dowling & Sidman’s (1962) observation of an over-production of rhodopsin-like protein. This apparent paradox was reconciled by the work of Lavail et al. (1972) who showed by radioautographic methods that the pigment epithelium layer of affected animals had an abnormally high rate of incorporation of labelled precursor amino acid into protein. This label was transferred to the lamellar protein which was deposited between the retina and the pigment epithelium. In addition it was shown that the normal retina produced visual pigment in the inner part of the photoreceptor cell which was transferred by displacement to the outer segment sacs. In the affected retina this synthetic process was decreased and the labelled protein only migrated into the basal third of the outer segments.

Farber & Lolley (1973) compared the protein patterns of retinae of mice with inherited blindness with those of normal mice by polyacrylamide-gel electrophoresis. In adult mice, the pattern from affected retinae was deficient in three bands of protein, two of which were opsin and the third one was cyclic AMP phosphodiesterase, thus confirming the observation of a decrease in specific protein synthesis in affected retinae.

It is therefore possible to define the sequential nature of the biochemical lesion and to carry out experiments to investigate the validity of this definition.

The pigment epithelium plays a major role in development of the degeneration. In the normal adult mammal, the relationship between the rod outer segments and pigment epithelial cells is expressed in at least two ways. First, derivatives of retinol cycle between the two cell types during the bleaching and regeneration process of visual pigment. Secondly, rhodopsin-containing discs from the outer rod segments become broken off at the distal end and their membranes become incorporated into the cytoplasm of the pigment epithelial cells by a process of phagocytosis. Effete protein of the discs once phagocytosed is digested by proteases contained in the lysosomes situated in the apical villi of the pigment epithelial cells. Lavail et al. (1972) showed that in the affected animal the pigment epithelial cells have an unusual synthetic role and are the source of the extralamellar material. This material appears to be identical with rhodopsin and is therefore broken down by light, releasing retinal, which is reduced to retinol. Because of the phagocytic function of the pigment epithelium, the cells are richly endowed in lysosomal particles; any build up of retinol in the pigment epithelium in the affected eye will tend to cause lysosomal activation, thus releasing acid proteases and hydrolases, accounting for the cellular digestion and the eventual complete disappearance of the neuroepithelium of the retina.
Evidence for the release of lysosomal enzymes was recently obtained in cytochemical and biochemical studies by Burden et al. (1971) and Yates et al. (1974). Increases in both total and free acid protease activity above those found in the normal retina were first observed in the affected retina at 4 weeks. These differences became more apparent as degeneration progressed. The stability of lysosomes in the pigment epithelium was studied by measuring the accessibility of the enzymes naphthylamidase and acid phosphatase in their respective substrates. It was found that in 1-week-old rats lysosomes in the affected pigment epithelium are less stable than those in normal tissue, the instability becoming more pronounced as the animals aged. These results support the idea that degeneration of visual cells is initiated by infiltration of lysosomal enzymes from the pigment epithelium.

The basic research which has been carried out on experimental animals has underlined the sequential nature of the degenerative changes but has also indicated that the underlying genetic abnormality is probably a multiple gene defect, since proteins other than opsin appear to be involved. However, the knowledge obtained holds out some hope for direct application in the clinical field. It may be possible to delay the release of lysosomal enzymes and preserve lysosomal stability in the pigment epithelium by the use of anti-inflammatory drugs. This has already been achieved to some extent by the use of acetylsalicylic acid to delay the onset of degeneration (Dewar & Reading, 1974a,b).


Biochemical Studies on the Dystrophic Rat Retina and Brain

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There is now sufficient information available on the mechanisms involved in hereditary retinal degeneration in the rat to enable tentative studies to be made into possible methods of retarding the progress of the degeneration. In the case of albino dystrophic rats (Campbells) there is evidence (Reading, 1974) to suggest that there is an abnormal build

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