Alcohol-Dehydrogenase Activity of the Retina in the Normal and Dystrophic Rat

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(Received 19 January 1966)

Hereditary degeneration of the retina in the rat first described by Bourne, Campbell & Tansley (1959). They held that before the degeneration the retina had become fully developed and that the retina was therefore 'abiotrophic' (i.e. premature of a normal tissue), a reading that fitted with a then widely held by ophthalmologists as to the nature of retinitis pigmentosa in man. In a study of a similar recessively inherited degeneration of the retina in the mouse, Sorsby, Koller, Attfield, & Lucas (1954) showed that the retina failed to reach maturity before the degenerative process set in, i.e. the lesion was a congenital failure of development with a postnatal course (dystrophy). The findings and interpretation were shown to be also to the rat (Lucas, Attfield & Davey, 1955) and to the dog (Lucas, 1954). Hereditary degeneration of the retina in laboratory animals is therefore the nature of a failure in development. In the rat, degeneration of the outer segments of the visual cycle can be seen first about 12–14 days after birth, whereas full differentiation of the normal rod cells does not occur until 21–28 days after birth, by which time the degeneration in the abnormal retina is very evident.

Chemical investigations of the condition have been chiefly concerned with comparisons of glucose metabolism in normal and affected tissues. Gray, Tansley & Kerly (1959) found anaerobic glycolytic activity of the dystrophic retina to be largely unaltered from the normal during the first weeks after birth and they were unable to show any relationship between glycolytic activity and visual-cell degeneration. Walters & Brotherton (1960) reported a decline in aerobic glycolysis in affected retinæ but not after 16–25 days of age. Reading & Sorsby (1964) found no qualitative differences in aerobic metabolism via the Embden-Meyerhof and glycolytic pathways between normal and dystrophic retinæ up to the age of 30 days; such quantitative differences that were detectable occurred well after histological degeneration had set in.

In contrast, studies on protein synthesis have given suggestive results. Reading & Sorsby (1964) found a decrease in the rate of protein synthesis as measured by the incorporation of [^{14}C]glycine in the

very young dystrophic retina (6–10 days), i.e. before the appearance of any histological anomalies. Subsequently, Reading (1964) also showed the hexose monophosphate pathway of glucose catabolism to be more active in the dystrophic than in normal retinæ. An elevation in activity by as much as 41% was evident, again at a very early age, namely at 6 to 7 days. Increases of 49 and 52% respectively were also present at 14 and 27–28 days. The hexose monophosphate pathway of glucose catabolism declined in relative importance compared with the glycolytic pathway with increase in age and cellular differentiation in both normal and dystrophic retinæ.

The hexose monophosphate pathway has been implicated in the normal functioning of the visual cycle in the mammalian retina (Futtermann, 1963). After bleaching of the visual pigment, liberated retinal (vitamin A aldehyde) is reduced to retinol (vitamin A alcohol) by NADPH_2 under the influence of the enzyme alcohol dehydrogenase (retinene reductase). The NADP thus formed then stimulates glucose oxidation by the dehydrogenases of the hexose monophosphate pathway. This pathway is active in the light-stimulated retina but quiescent in the dark, and its activity is influenced by the availability of NADP.

Beaconsfield & Reading (1964) showed that intrinsic hexose monophosphate shunt activity in a number of tissues can be depressed by increases in activity of the NADP-linked alcohol-dehydrogenase system and that the two enzyme systems are 'coupled' in an inverse manner. Since hexose monophosphate pathway activity was found to be relatively high in the dystrophic retina, even before histological changes were discernible, the present study was devoted to assessing alcohol-dehydrogenase activity during the period of development of the lesion in the dystrophic retina.

Methods. The affected rats were descendants of the original strain of pink-eyed piebald agouti rats (Bourne *et al.* 1938), and for comparison normal black hooded (PVG) rats were used.

All experiments were carried out on litter mates irrespective of sex. Rats were killed by cervical fracture, the eyes enucleated, and retinæ prepared for experiments by dissection under ice-cold 0.9%

NaCl. Wet weights of retinal tissue were determined on a torsion balance after removing surplus liquid.

Alcohol-dehydrogenase activity was estimated in 0.25M-sucrose homogenates of pooled rat retinae (made in a Teflon homogenizer), which were incubated at 37° in flasks in a Warburg apparatus to ensure uniformity. Incubation mixtures consisted of 1ml. of 0.25M-sucrose homogenate of retinae, 750 μ g. of vitamin A alcohol (pure crystalline; Eastman Kodak Co., Rochester, N.Y., U.S.A.) dispersed in 0.1ml. of 10% (w/v) Tween 80 in acetone, 2mg. of NAD (British Drug Houses Ltd., Poole, Dorset) in 0.1ml. of 0.1M-glycine buffer, pH 9.6, and 1.8ml. of 0.1M-glycine buffer, pH 9.6. Samples (0.5ml.) were withdrawn at zero time and after incubation for 30 and 90 min. The retinal content of these samples was estimated by the thio-barbituric acid method of Futterman & Saslaw (1961). Colour development in the reaction mixtures was measured at 530m μ with a Beckman model DU spectrophotometer. Suitable controls containing no enzyme (retina) and no coenzyme were included in estimations together with reagent

blanks and standard retinal (Eastman Kodak solutions. Unless otherwise stated all reagents of A.R. grade.

All estimations were carried out on 6–12 and each value is the mean of three or four estimations.

Results. Alcohol-dehydrogenase activity negligible in both normal and dystrophic retina until 6–8 days after birth. A tendency for dystrophic retinae to show slightly higher activity than the normal at 6–8 days should be ignored, the enzyme estimation at such an early age complicated by the small quantity of tissue available and by very low rates of substrate conversion.

In the normal rat retina, alcohol-dehydrogenase activity increased steadily until the animals about 1 month old, this period being followed by levelling off until a steady state of activity was reached in the adult (Fig. 1). This followed a pattern of a normal growth–development curve.

The enzyme in the dystrophic retina developed similarly until the animals were about 2 weeks whereupon wide variations in activity were seen with some evidence of an elevated activity at 16–18 days of age (Fig. 1). Subsequently, however, a marked decrease in activity was apparent from 3 weeks onwards, until retinae from 4-week dystrophic rats possessed only 40% of the enzyme activity of the corresponding normal animals.

Discussion. In view of the increased activity of the hexose monophosphate pathway in the dystrophic rat retina and the ‘coupling’ of this pathway with that of the alcohol-dehydrogenase system in retina and other tissues and its relation to the visual cycle, it was suggested (Reading, 1965) that alcohol-dehydrogenase activity might be decreased or absent from the dystrophic retina and such a decrease could conceivably cause a breakdown in the dynamics of visual-pigment synthesis and bleaching, with ensuing cellular degeneration. Though the results of the present series of experiments support the original suggestion to a certain extent, the depression of alcohol-dehydrogenase activity in the retina did not manifest itself until after the third week of life, the enzyme showing appreciable activity at 18–20 days of age, a stage at which the dystrophic retina showed substantial degenerative changes in the outer segments. The decline in alcohol-dehydrogenase activity in the retina can therefore be regarded as consequential to the primary biochemical changes causing cellular degeneration.

We thank Miss Diane Hadlow for technical assistance, the National Council to Combat Blindness, New York for a ‘Fight for Sight’ award of the spectrophotometer in this work.

Fig. 1. Alcohol-dehydrogenase (retinene reductase) activities of the young developing normal and dystrophic rat retinae. The enzyme was assayed in sucrose homogenates of rat retinae by determination of the rate of production of retinal (vitamin A aldehyde) from retinol (vitamin A alcohol) in the presence of NAD. ●—●, Normal (PVG) rat retinae; △—△, retinae from rats affected with hereditary retinal degeneration (dystrophy).

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J. (1966) **99**, 5c

An Improved Analytical Procedure for Automatic Analysis of Amino Acids

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(Received 25 January 1966)

There is an increasing need for rapid and precise determination of amino acids. Hamilton (1960), and Hamilton, Bogue & Anderson (1960) have identified the critical factors affecting the chromatographic separation of amino acids. By using beads of small and uniform size, narrow-bore columns and an eluting gradient it is now possible to separate all the amino acids in a protein hydrolysate. Column resolution is so sharp that many of the amino acids are eluted as peaks of no more than 0.5 min. duration.

Good resolution is a product of both chromatographic and analysis, and though much attention has been paid to column resolving power there has been comparatively little development of the analytical system. In the automated analytical system most commonly used, the column effluent is mixed with the reagent (ninhydrin/hydrindantin 10% methylCellosolve heavily buffered) and heated at 95° in a long-time-delay coil to allow for colour development (Piez & Morris, 1960; Spackman & Moore, 1958). Diffusion is minimized by using a long capillary coil or by segmenting the coil with oxygen-free nitrogen. We have used a new method with the Technicon Autoanalyzer. In this system a steady concentration of an amino acid is maintained throughout this analytical system, we have found that 5 min. is required for the full colour to be attained and over 1 min. for the half-maximum value to be reached. There is an equilibrium on instantaneous withdrawal of the effluent. This slow rise time seriously impaired

the overall resolution of the chromatogram. We attribute this slow rise time to the presence of methylCellosolve, since similar wholly aqueous systems have a much more rapid response. A slow rise time of this kind will decrease the resolution of closely adjacent peaks even on a slow (22 hr.) chromatogram. A further disadvantage of analytical procedures that use hydrindantin is the extreme sensitivity to oxygen. The reagent is therefore difficult to prepare and not easily stored. It also necessitates the rigid exclusion of air from the entire system, a condition not easy to satisfy adequately when using gradient elution, particularly with the high pumping pressures required with fast chromatograms. We have repeatedly found a random variation in colour yield in different parts of the chromatogram. This we attribute to air dissolved in the buffers at the high pressures used. A high background colour is also a significant feature.

It would be clearly desirable to have an analytical system that was insensitive to oxygen and containing little or no methylCellosolve and consequently showing a faster rise time. We experimented with the trinitrobenzenesulphonic acid method suggested by Hochstrasser (1965), but in addition to being insensitive to proline and hydroxyproline, which have to be determined separately, the colour yield was only about one-fifth of that of the ninhydrin system. Cadavid & Paladini (1964) described a ninhydrin method adapted from that of Rosen, Berard & Levenson (1962) in which added hydrin-