Retinol and Retinyl Esters in Pigment Epithelium of Rats with Inherited Retinal Degeneration

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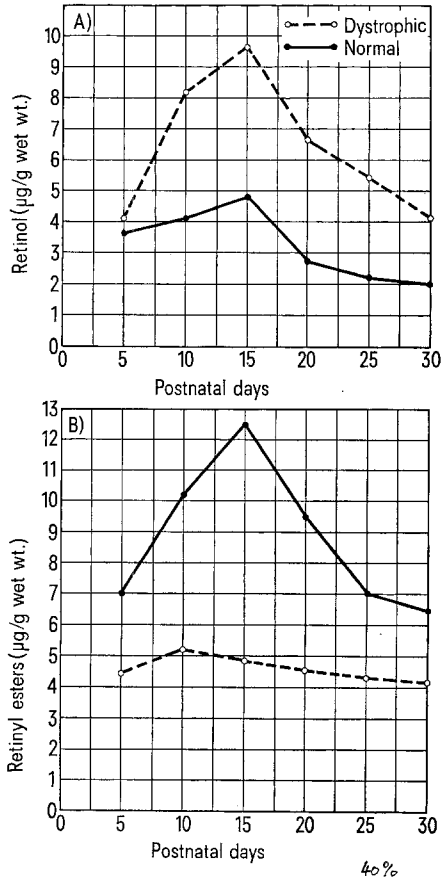
Summary. A comparative study of the retinol and retinyl ester concentrations was performed in the retinal pigment epithelium of the normal and affected rats. Our findings indicate that in dystrophic rat retinol content increases, whereas the amount of retinyl esters is always lower than normal. An hypothesis can be made on the deficiency of enzymic activities which regulate retinol retinol and retinyl esters levels in the pigment epithelium.

Inherited retinal dystrophy in the rat is transmitted by an autosomal recessive gene. The disease can be seen about 12–15 days after birth, as a result of degeneration of the photoreceptor cells[1–3]. The primary significant biochemical lesion of the retinal degeneration in the rat has been shown by Reading[4], who found in affected rats at 21 days of age that, after light adaptation, the amount of retinol in the pigment layers of the dystrophic eye is at least twice that in the normal rat of corresponding age. Therefore it has been postulated that the increased amount of retinol in the dystrophic pigment epithelium could labilize the lysomes in this tissue, the lysosomal enzymes being released into the pigment epithelium and adjoing visual cell layers, with ensuing cellular breakdown[5–7]. Some hypothesis can be made to explain the comparatively high concentration of retinol in the dystrophic pigment epithelium. We considered that the increase in retinol in the dystrophic pigment epithelium could result from the variation of enzymatic activities closely related to metabolism of retinol in the retina and pigment cell layers. Alcohol dehydrogenase[8–10] and retinol esterase[11] could play a role in controlling the levels of retinol in both these tissues. However, no differences in alcohol dehydrogenase activity are reported by Reading[12] in the normal and dystrophic rat until the 3rd week of life, and the following decline in alcohol dehydrogenase activity in the retina can therefore be regarded as consequential to cellular degeneration and not part of the primary biochemical lesion. We decided to measure, in the pigment epithelium of dystrophic rats, levels of retinol and retinyl esters at various ages. The distribution of retinol and retinyl esters was therefore compared in normal and affected eyes in conditions under of room light adaptation.



Retinol and retinyl esters concentration during development in retinal pigment epithelium layers of normal and affected rat. Points represent mean ± SD of 5 determinations.

Materials and methods. The affected rats were descendents of the strain pinked-eyed piebald agouti rats, and for comparison normal black hooded (PVG) rats were used. Normal and affected rats, aged 5, 10, 15, 20 and 30 days, were killed by decapitation. The eyes were removed, sectioned and the retinas separated from the tissues of the back of the eye. 30 cups from each age were homogenized in 5 ml of 0.15 *M* NaCl and homogenate extracted with 20 ml of a mixture containing *n*-propanol, petroleum ether and water (5:3:8). The extract, evaporated under N_2, taken up in a small volume of petroleum ether and applied to column of alumina weakened by addition of water 5% (w/w), was eluted by the Gangly procedure[13]. Retinol and retinyl esters, separated chromatographically, were assayed by the Carr-Price reaction[14].

Results and discussion. Retinol in the normal pigment epithelium increases postnatally, reaching a maximum value after 15 days (Figure A). In the developing pigment cell layers of affected rats, the content of retinol was normal for 5 days and then it increased abnormally, reaching a peak value at days 14 to 15. Subsequently, at 20 days, retinol decreased. The retinyl esters content of the normal retinal pigment epithelium increased rapidly after birth, reaching near adult values by 15 days of postnatal age. By comparison, the retinyl esters content

[1] M. C. Bourne, D. R. Campbell and K. Tansley, Br. J. Ophthal. *22*, 613 (1938).
[2] D. G. Cogan, Trans. Am. Acad. Ophthal. Otolaring, 54th Meeting (1950), p. 629.
[3] D. R. Lucas, J. exp. Zool. *126*, 537 (1954).
[4] H. W. Reading, Biochem. J. *100*, 34 (1966).
[5] E. M. Burden, C. M. Yates, H. W. Reading, L. Bitenski and J. Chayen, Expl Eye Res. *12*, 159 (1971).
[6] R. Vento and F. Cacioppo, Expl Eye Res. *15*, 43 (1973).
[7] A. J. Dewar, G. Barron and H. W. Reading, Expl Eye Res. *20*, 63 (1975).
[8] G. Wald and R. Hubbard, J. gen. Physiol. *32*, 367 (1949).
[9] T. Amemiya, Acta Soc. ophthal. jap. *71*, 2115 (1967).
[10] C. Nicotra and M. A. Livrea, IRCS med. Sc. *3*, 306 (1975).
[11] N. Krinsky, J. biol. Chem. *232*, 881 (1958).
[12] H. W. Reading and A. Sorbsy, Biochem. J. *99*, 30 (1966).

of the dystrophic pigment epithelium was below that of the normal pigment epithelium at age 5 days, after which time it was steadily lower than normal (Figure B).

To conclude, our findings show that in affected rats aged 10 to 15 days (which coincides with the postanatal period when cell death occurs most rapidly) retinol content increased, whereas the amount of retinyl esters was apprecialby reduced. The decrease of retinyl esters content in epithelium cell layers could be the consequence of a defective or absent retinol esterifying activity. It is probable that, in the diseased eye, not only the protein pattern of the retina is defective[15] but the pigment epithelium may also lack some enzymatic proteins. Retinal hereditary degeneration related to a deficiency of several proteins would be then caused by multigenetic defect.

[13] J. GANGULY, N. I. KRINSKY, J. W. MEHL and R. J. DEUEL JR., Arch. Biochem. Biophys. *38*, 275 (1952).
[14] O. A. ROELS and S. MAHADEVAN, in *The Vitamins* (Eds. P. GYORGY and W. N. PEARSON; Academic Press, New York 1967), vol. 6, p. 155.
[15] D. B. FARBER and R. N. LOLLEY, J. Neurochem. *21*, 817 (1973).

Purification of Plasminogen Activator(s) from Human Seminal Plasma[1]

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Summary. Human seminal plasma contains an activator of plasminogen which was chromatographically purified. The molecular weight was determined to be 67,000.

The conversion of plasminogen into plasmin can be effected by various naturally occurring activators, such as tissue activator, blood activator, urine activator (urokinase) and trypsin. Also, in seminal plasma, an activator of plasminogen has been found[2–6]. Apart from its physiological significance, it is also of interest in the search for thrombolytic enzymes.

This paper concerns chromatographic purification and characterization of the plasminogen activator in human seminal plasma.

Material and methods. Semen samples were obtained from the Fertility Laboratory. They were centrifuged at 15,000 *g* for 1 h and the supernatant was decanted. 60 ml of the clear supernatant was dialysed in 3 × 2 l, 0.1 *M* acetate buffer, pH 5.0 for 3 × 12 h. A precipitate formed, which was removed by centrifugation at 15,000 *g* for 1 h. This procedure was repeated.

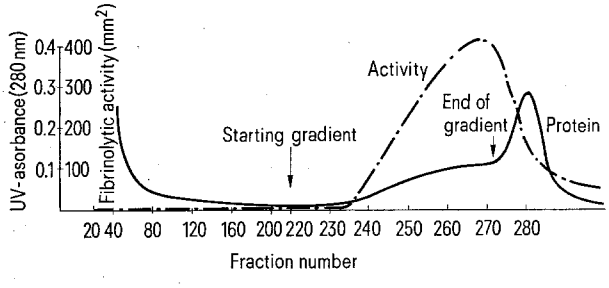

Fig. 1. Elution profile of seminal plasma on CM-Sephadex C-50 (see text).

The dialyzed seminal plasma with a volume of 100 ml was made 0.2 *M* in NaCl. Ionic exchange chromatography was then performed on a column of CM-Sephadex C-50, 4.4 × 10 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.1 *M* acetate buffer, pH 5.5, 0.2 *M* NaCl. The column was first eluted with starting buffer and then a linear NaCl-gradient was applied (0.2–0.5 *M* NaCl). Fractions of 15 ml were collected at a flow rate of 60 ml/h. The absorbancy of each fraction was read at 280 nm. The fibrinolytic activity of the fractions was tested on unheated and plasminogen-free fibrin plates[7]. The fibrinolytically active fractions were concentrated by ultrafiltration (Diaflo ultrafiltration cell, membrane PM 10) prior to gel filtration.

Gel filtration was performed on a column of Sephadex G-200, 2.5 × 100 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.02 *M* *Tris*-HCl, pH 7.4, 0.3 *M* HCl. 5 ml of the concentrate was applied. Fractions of 5 ml were collected at a flow rate of 18 ml/h. The absorbancy and fibrinolytic activity were determined as previously described.

The molecular weight was measured on a column of Sephadex G-100, 2.5 × 100 cm (Pharmacia Fine Chemicals, Uppsala, Sweden), in 0.05 *M* *Tris*-HCl, pH 7.4, 0.1 *M* HCl. The column was calibrated with Blue Dextran (V_o),

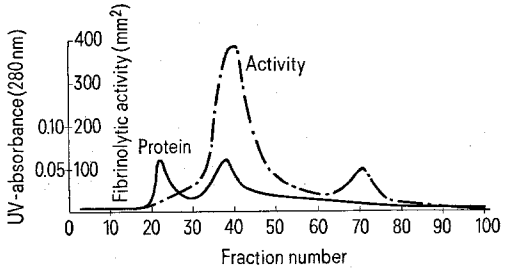

Fig. 2. Elution profile of seminal plasma on Sephadex G-200 (see text).

[1] This study was supported by grants from the Swedish Medical Research Council (No. B75-17X-4523-01).
[2] K. N. VON KAULLA and L. B. SHETTLES, Proc. Soc. exp. Biol. Med. *83*, 692 (1953).
[3] F. LUNDQUIST, TH. THORSTEINSSON and O. BUUS, Biochem. J. *59*, 69 (1955).
[4] J. RASMUSSEN and O. K. ALBRECHTSEN, Fertil. Steril. *11*, 264 (1960).
[5] P. LIEDHOLM and B. ÅSTEDT, Fertil. Steril. *24*, 517 (1973).
[6] L. J. D. ZANEVELD, G. F. B. SCHUMACHER, P. F. TAUBER and D. PROPPING, Bayer-Symposium V *Proteinase Inhibitors* (Springer Verlag, Berlin 1974), p. 136.
[7] I. M. NILSSON and B. OLOW, Thromb. Diath. haemorrh. *8*, 297 (1962).