ATPase in the Normal and Dystrophic Developing Retina of the Rat

This communication deals with preliminary data on ATPase (ATP phosphohydrolase) activity in the developing retina of 2 normal rat strains and a strain with inherited retinal degeneration[1]. Since ATPase has been implied in the Na^+–K^+ transfer at cellular membranes[2], the opportunity to investigate the stimulation by Na^+ and K^+ ions of ATPase activity also in the dystrophic retina lacking a normal electroretinogram[3] is most obvious. The electroretinographic pattern (ERG) appears in the normal retina only after a certain developmental stage[3], and the alteration of the ERG is one of the first dynamic signs of degeneration in the visual cells[3]. The overall picture obtained by measurements of ATPase in the presence and absence of Na^+ and K^+ ions has been in keeping with the hypothesis of a post-natal decrease in the Na^+–K^+ activation of ATPase in the dystrophic retina.

Albino rats of the Wistar and Sprague-Dawley strain, 2–120 days old, were used as controls. Rats with retinal dystrophy, descendants of the strain described by BOURNE et al.[1], were also used. The cornea, iris and lens were gently removed, the eye-ball was immersed in 0.25 *M* sucrose at 2–4 °C, and the retina was separated from pigment epithelium and choroid. Histological examination of retinae isolated by this technique has shown the presence of only a few fragments of choroid. However, in the affected strain the isolation of retina from pigment epithelium was not so easy as in the normal, owing to the adhesion of the 2 structures.

Soon after removal, 16–20 retinae were extracted with glass distilled water by homogenization with a motor-driven glass homogenizer in a final volume of 1.5 ml. The homogenate was centrifuged at 22,000 *g* for 20 min in a Lourdes refrigerated centrifuge. All steps were performed at 4 °C. When young developing rats or animals of the affected strain were used, the number of retinae in each experiment was greater.

ATPase activity has been measured in the experimental conditions suggested by SKOU[2] and protein nitrogen has been determined by a submicrospectrophotometric procedure[4].

Tris (hydroxymethyl)-aminomethane-ATP was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). The other compounds were reagent grade.

The Table summarizes data indicating a definite increase in the specific activity of ATPase in the presence of Mg^{++} or Mg^{++} plus Na^+ and K^+ ions during the post-natal development of retinae from the normal and affected rat strains. However, Na^+-K^+ stimulation of ATPase activity does not vary in the normal retina, while it declines in the degenerating tissue. The Figure shows that a decline of Na^+-K^+ activation can be measured much earlier than the 22nd day of post-natal life, a decrease of the ratio Mg^{++}–Na^+–K^+/Mg^{++} stimulated ATPase activity being already apparent by the 12th day after birth. It is also interesting to underline that the ratio between Mg^{++}–Na^+–K^+/Mg^{++} ATPase activity does not change in the normal developing retina. A value of 1.8–2.2 has been obtained for this ratio at all developmental stages of the normal retina, under the experimental conditions reported above.

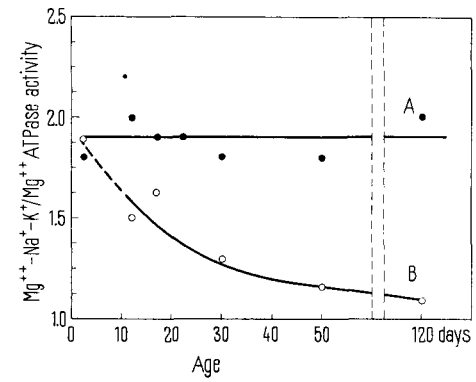
As suggested by SKOU[2], the cleavage by ATPase of the terminal $\sim P$ bond of ATP releases the energy required for the entrance of K^+ and the extrusion of Na^+ through cellular membranes after depolarization. BONTING et al.[5] have recently adapted this general theory to the visual cells. If it is true that light generates a current which flows from the photoreceptor cell body to the outer segment[6], it follows that a movement of ions occurs through the receptor membrane upon light excitation, just as it occurs in the nerve. The Na^+–K^+ ATPase system would then operate in the repolarization process to pump out Na^+ and move K^+ back in (see also [7]).

As mentioned above, the dystrophic retina lacking a normal electroretinogram has appeared as a system which might reveal significant changes of the ATPase system. Experimental findings have been in keeping with the hypothesis. However, there is another finding obtained in ATPase measurements which deserves a short comment. The immature retina of a normal rat strain does not exhibit the electroretinogram up to the 12th day of post-

ATPase specific activity (activity/mg protein) in the developing retina[a]

Age (days after birth)	Normal retina[b]		Dystrophic retina	
	Plus Mg^{++}	Plus Mg^{++}-Na^+-K^+	Plus Mg^{++}	Plus Mg^{++}-Na^+-K^+
2	3.6	6.5	3.5	6.6
120	7.2	14.3	7.9	8.8

[a] For experimental details see text. ATPase specific activity has been expressed as micromoles of *P*/min/mg protein at 37 °C. [b] Data reported in the Table and the Figure for normal retinae were obtained on albino rats of the Wistar strain. No significant differences were found, however, between retinae of Wistar and Sprague-Dawley strains.



The ratio between ATPase activity in the presence of Mg^{++}-Na^+-K^+ and ATPase activity after addition of only Mg^{++} ions is reported as a function of post-natal development of normal (A) and dystrophic (B) retina. Each experimental point represents the mean of at least 3 determinations in duplicate on pools of 16 retinae or more.

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natal life[3]. Nevertheless, the enzyme system responsible for ATP cleavage and energy disposal does exhibit the same properties found in the adult retina, no changes in the ratio Mg^{++}–Na^+–K^+/Mg^{++} stimulated ATPase activity being measured during the first 4 months of life. CÔTÉ[8] has shown that the enzyme activity is measured in the brain before the occurrence of electrical activity. Though the alteration of the ERG in the affected animals is easily related to the damage of photoreceptor cells, it is worth noting that the early decline of Na^+-K^+ stimulation of ATPase activity offers a suggestive dynamic explanation of the transitory appearance and subsequent disappearance of the electroretinogram in this same rat strain[3]. One could suppose that the ERG appears because at the 12th day ATPase is still largely stimulated by Na^+ and K^+ ions and declines soon after, since the ionic stimulation of enzyme activity becomes insufficient. If ATPase is an allosteric enzyme, as suggested by SQUIRES[9], a conformational alteration of the catalytic protein at the allosteric site for Na^+ and K^+ ions would be responsible for the observed changes in ATPase activities. Whatever the possible speculations, it can only be stated that ATPase stimulation by Na^+ and K^+ ions declines in the dystrophic retina with age in comparison with non-Na^+–K^+ stimulated ATPase[10].

Riassunto. Viene descritto un aumento significativo dell'attività specifica dell'ATPasi (attività/mg proteine) durante lo sviluppo post-natale della retina in ratti normali ed in ratti con retinite pigmentosa ereditaria. Il rapporto tra attività ATPasica in presenza di Mg^{++}–Na^+–K^+ ed attività ATPasica in presenza di Mg^{++} non varia nella retina normale, mentre esso va incontro ad un precoce declino durante lo sviluppo della retina distrofica. Il significato di questi dati è discusso molto brevemente.

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The Production of Leucylphenylalanine Anhydride by a Variant of *Streptomyces noursei*

Streptomyces noursei, variant No. 5286, which produces the antibiotic phalamycin[1] also produces several substituted dioxopiperazines[2]. Four of them have been reported, namely: 3,6-dibenzylidene-2,5-dioxopiperazine (I), its dihydro derivative 3-benzyl-6-benzylidene-2,5-dioxopiperazine (II), and its tetrahydro derivative 3,6-dibenzyl-2,5-dioxopiperazine (III), and 3-benzylidene-6-isobutylidene-2,5-dioxopiperazine (IV). Because of the relationship between I, II, and III a subsequent search was made for further products which might be similarly related to IV by saturation of either or both of the olefinic double bonds. Only the fully saturated product, namely 3-benzyl-6-isobutyl-2,5-dioxopiperazine or leucylphenylalanine anhydride (V) was found. This note reports its isolation and proof of identity by methods previously described[2].

For the isolation, ethyl acetate extracts of 4-day shake cultures in yeast extract broth were concentrated to dryness in vacuo and the residue extracted repeatedly with each of the following solvents in succession: water, and 10, 20, 30, 40, 50, 60, 70, 80, and 90% acetone. Based on IR-spectra, pools were made and solids obtained by concentration in vacuo. In the 70–90% acetone extracts there was evidence for IV as well as V. In the 10–60% acetone extracts there was strong evidence (in particular, 3 characteristic absorption bands at 1347, 1336, and 1325 cm^{-1}) for the presence of V. These extracts contained also much highly colored material, most of which could be removed by several washings with ether. The residue was further purified by repeated sublimations at a temperature of approximately 180 °C and a pressure of about 1 mm Hg. Waxy resinous solids collected on the cold finger in the early cuts of this process, and clean white amorphous solids sublimed thereafter. Efforts to crystallize this latter material revealed a striking difference from the other dioxopiperazines. Crystallization could not be initiated or was practically negligible from most of the ordinary organic solvents or solvent pairs; either a stiff gel formed when the hot solutions were cooled, or no crystals appeared if the solutions were dilute enough to avoid gel formation. From aqueous acetic acid, approximately 60%, long fibrous, glistening white crystals were sometimes obtained after several days at – 5 °C, but only in very low yield. However, when crystals failed to appear from the acetic acid-water solutions or when a gel formed, a moderately good yield of crystals could be obtained by first freezing the solution or gel and then thawing it at room temperature. The tiny white rod-shaped crystals thus formed melted a few degrees lower than the larger crystals mentioned above, but nevertheless this step brought about considerable improvement in purity.

This natural V was identified by comparison of its properties with those of the synthetic product prepared by cyclization of L-leucyl-L-phenylalanine. Both natural and synthetic products show the same principal absorption maxima: 3210, 3070, 2960, 2905, 1665, 1605, 1499, 1465, 1458, 1390, 1370, 1347, 1336, 1325, 1093, 1012, 912, 868, 836, 766, 752, and 700 cm^{-1}. The natural product melts with decomposition at 272–273 °C, the synthetic at 273–274 °C, and the mixture melting point shows no depression. Analysis of the natural substance gave: C, 69.46; H, 7.52; N, 10.65; calculated for $C_{15}H_{20}N_2O_2$: C, 69.18;

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