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Harderian Gland. V.[1] Effect of Dietary Pantothenic Acid Deficiency on Porphyrin Biosynthesis in Harderian Gland of Rats

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The correlation between the chromodacryorrhea and the excess porphyrin formation by the Harderian gland of pantothenic acid-deficient rat was examined. It was thereby found that the main porphyrin in the red tears on pantothenic acid-deficient rats was not coproporphyrin, reported by McElroy, *et al.*, but was protoporphyrin-IX, thin-layer chromatographycally and spectrophotometrically. According to the measurement of porphyrin content and δ-aminolevulinic acid synthetase (succinyl-CoA: glycine C-succinyltransferase (decarboxylating), EC 2.3.1.37) activity in the Harderian gland, it was assumed that the chromodacryorrhea was caused by secretion of excess protoporphyrin formed due to the increase in δ-aminolevulinic acid synthetase activity and the hypertrophy of Harderian gland.

It is now well established that the Harderian gland of rat has the biosynthetic pathway from δ-aminolevulinic acid to protoporphyrin-IX.[1,3] On the Harderian gland of rat, deficiency of pantothenic acid results in the secretion of porphyrin from eyes or the flow of red tears (chromodacryorrhea),[4] and major component of these porphyrins was identified as coproporphyrin.[5]

In order to investigate the correlation between chromodacryorrhea and the porphyrin formation in the Harderian gland of pantothenic acid-deficient rat, the identification of the porphyrin in red tears was carried out and it was found that the major component of the porphyrin was not coproporphyrin but was protoporphyrin-IX.

This paper presents that the chromodacryorrhea is due to the excessive formation of protoporphyrin-IX by the increase in δ-aminolevulinic acid synthetase (succinyl-CoA: glycine C-succinyltransferase (decarboxylating), EC 2.3.1.37) which is a rate-limiting enzyme in the biosynthetic pathway of porphyrin,[6] and by the hypertrophy of Harderian gland.

Materials and Method

Animals——Young male rats (30—40 g) of Wistar strain, immediately after weaning, were kept in the metal cages designed so as to prevent the coprophagia in the room air-conditioned at 22—25° until the ap-

1) Part IV: K. Eida and M. Kikutani, *Chem. Pharm. Bull.* (Tokyo), **17**, 927 (1969).
2) Location: *Bunkyo-cho, Nagasaki, 852, Japan.*
3) J.M. Tomio and M. Grinstein, *Eur. J. Biochem.*, **6**, 80 (1968).
4) S. Tashiro and H. Stix, *Biol. Bull.*, **64**, 327 (1935).
5) L.W. McElroy, K. Salomon, F.H. J. Figge, and G.R. Cowgill, *Science*, **94**, 467 (1941).
6) *a*) S. Granick and G. Urata, *J. Biol. Chem.*, **238**, 821 (1963); *b*) J. Hutton and S. Gross, *Arch. Biochem. Biophys.*, **141**, 284 (1970).

pearance of chromodacryorrhea. The symptom of chromodacryorrhea began on about 3rd week, with cessation of growth, on all of pantothenic acid-deficient rats. When the rats with chromodacryorrhea were viewed under ultraviolet light, deep fluorescence of porphyrin could be seen on the eyelids, nose, face, and fore paws.

Pantothenic acid-deficient diet was prepared by the method of Kuwagata.[7] The control animals were fed the diet containing pantothenic acid.

Materials——Harderian glands and livers were obtained from freshly killed rats by decapitation. δ-Aminolevulinic acid was purchased from the Daiichi Pure Chemical Co., Tokyo. Protoporphyrin-IX and coproporphyrin-III were prepared by the method of Grinstein[8] and of Rimington,[9] respectively. Silica gel used for thin-layer chromatography was Wakogel B-5 (Wako Pure Chemical Ind., Osaka). Other chemicals used were commercial products.

Preparation of Red Material——Red materials on the eyelids, nose, whiskers, and fore paws were eluted with a small amount of 1 N HCl respectively. The deeply pigmented solution was submitted to the following analysis.

Thin-Layer Chromatographic Analysis and Measurement of Absorption Spectrum of Red Material——Thin-layer chromatography devised in this laboratory was used. A mixture of 20 g of Wakogel B-5 and 50 ml of 0.5 N HCl was stirred, allowed to stand for 20 min, and the gel was washed thoroughly with water. The gel was further washed with acetone and dried overnight in a thermostatic dryer. The dried gel was evenly mixed with a slight excess of 2 volumes of water by vigorous shaking and made into a thin-layer. The sample dissolved in 1 N HCl was applied to the starting line of this plate. The plate was developed by ascending technique with a solvent system of 0.5 N HCl–dioxane (15 : 1) until the solvent front migrates about 10 cm, at 20°.

After development the plate was air dried, and the spot of porphyrin was detected under ultraviolet light (Manaslu Light, long wave, 3650Å). Each spot was removed from the appropriate places and porphyrins were eluted with the solvent (acetone:1 N HCl=10:1). The eluant was separated by centrifugation and then evaporated to dryness *in vacuo*. The residue was dissolved in 1 N HCl solution. Porphyrins were identified by the *Rf* values and by measurement of the Sorêt maxima with a Beckman DB recording spectrophotometer.

Measurement of Porphyrin Content of Harderian Gland——To *ca.* 800 mg of the Harderian gland, 10 ml of a 1 : 1 buffer-saline solution was added and the mixture was homogenized in a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 2500 rpm for 10 min. An aliquot of the supernatant was placed in a test tube and conc. HCl was added to make the final concentration of 25% HCl solution. The absorbance of this solution was measured at 412 nm, using a Beckmann DB recording spectrophotometer. Protein concentration was determined by the modified Folin method of Lowry, *et al.*[10]

Measurement of δ-Aminolevulinic Acid Synthetase Activity——Measurement of δ-aminolevulinic acid synthetase activity was carried out by a slightly modified method of Marver, *et al.*[11] with the Harderian gland and the liver, which were homogenized in 10 volumes of 0.9% sodium chloride solution containing 0.5 mM EDTA and 10 mM Tris-HCl, pH 7.4, to the Harderian gland and 3 volumes of the same buffer to the liver respectively.

Result

Thin-layer chromatogram of the red material extracted from the red tears of pantothenic acid-deficient rats is shown in Fig. 1. It was found that the red tears taken from any place on the face of rat contain the same material with *Rf* value, 0.14—0.18, which is the same as that of protoporthyrin-IX, as shown Fig. 1 and Table I.

Table II shows that the porphyrin content in the Harderian gland of pantothenic acid-deficient rats is higher than that in normal gland.

For the elucidation of this higher content of porphyrin during pantothenic acid-deficiency period, examinations were made on the activity of δ-aminolevulinic acid synthetase, a rate-limiting enzyme in the porphyrin biosynthesis pathway. The activity of δ-aminolevulinic acid synthetase in the liver and the Harderian gland of pantothenic acid-deficient rats is

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8) M. Grinstein, *J. Biol. Chem.*, **167**, 515 (1947).
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11) *a*) H.S. Marver, D.P. Tschudy, M.G. Perlroth, and A. Collins, *J. Biol. Chem.*, **241**, 2803 (1966); *b*) H.S. Marver, D.P. Tschudy, M.G. Perlroth, A. Collins, and G. Hunter, Jr., *Anal. Biochem.*, **14**, 53 (1966).

presented in Table III. The ratio of δ-aminolevulinic acid synthetase activity in the Harderian gland of pantothenic acid-deficient rats to that of control rats is about 2.1: 1. This may explain the higher value in porphyrin content in the Harderian gland of pantothenic acid-deficient rats.

Discussion

In order to resolve biochemically the chromodacryorrhea, reëxamination was carried out, and the red tears obtained from various places on the face of the rat were analyzed separately. The thin-layer chromatography devised in our laboratory was very successful for the qualitative estimation of porphyrin, because free porphyrin can be applied *per se*, without deriving it to its methyl ester. It was found that every main porphyrin in the red tears was not coproporphyrin but protoporphyrin-IX. This suggests that the secretion of red tears is not due to an inhibition of conversion from coproporphyrinogen to protoporphyrin-IX.

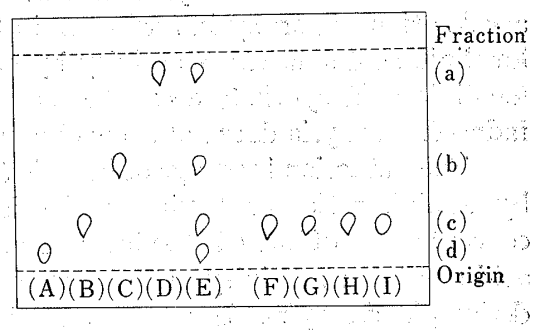

Fig. 1. Thin-Layer Chromatogram of Authentic Porphyrins and Red Tears of Pantothenic Acid-Deficient Rat on Silica Gel (Wakogel B-5)

Plate of Silica gel was developed with a solvent systems of 0.5N HCl-dioxane (15: 1) until the solvent front moved about 10 cm, at 20°, the development time required to be about 18 min.
(A): authentic protoporphyrin-IX methyl ester
(B): authentic protoporphyrin-IX
(C): authentic coproporphyrin-III
(D): authentic uroporphyrin
(E): mixture of four authentic porphyrins
(F): red material on eyelids
(G): red material on nose
(H): red material on paws
(I): red meterial on whiskers

TABLE I. *Rf* Values and Sorêt Maxima of Porphyrins

Fractions	*Rf*[a]	Sorêt maxima[b] (nm)	Porphyrins
(a)	0.77—0.90	406	uroporphyrin
(b)	0.46	401	coproporphyrin-III
(c)	0.14—0.18	408	protoporphyrin-IX
(d)	<0.10	407.5	protoporphyrin methyl ester

a) thin-layer chromatography with Silica gel (Wakogel B-5); development in 0.5N HCl-dioxane, 15:1 (v/v)
b) measured in 1N HCl solution

TABLE II. Effect of Pantothenic Acid-Deficiency on Porphyrin Content in Harderian Gland[a]

Treatment	Porphyrin content ((μg/mg protein)
Control	28.3± 4.7
Pantothenic acid deficiency	39.2±11.6

a) The data are presented as mean±S.E. of the results obtained with eleven rats (control) or fifteen rats (pantothenic acid-deficiency).

TABLE III. Effect of Pantothenic Acid-Deficiency on δ-Aminolevulinic Acid Synthetase Activity[a]

Tissues	Treatment	δ-Aminolevulinic acid synthetase activity (nmol/g/hr)
Liver	control	21.8±11.6 (8)
	pantothenic acid-deficiency	34.8±12.1 (6)
Harderian gland	control	122.1±27.2 (8)
	pantothenic acid-deficiency	265.0±25.0 (6)

a) The data are presented as mean±S.E. The number of rats in each group is given parentheses.

Many reports have revealed that porphyria is the abnormal metabolism due to an increase in δ-aminolevulinic acid synthetase activity. Our experimental results showed that a marked acceleration of porphyrin formation in the Harderian gland is due to the increase in δ-aminolevulinic acid synthetase activity by pantothenic acid-deficiency. Though hepatic δ-aminolevulinic acid synthetase can be induced by various chemicals,[12] the rat with chemically induced porphyria does not show chromodacryorrhea.[13]

The induction by drugs of hepatic δ-aminolevulinic acid synthetase is strongly suppressed by administration of hemin[14] which is the actual regulatory agent in the liver. Since the concentration of heme is too low in Harderian gland to influence the level of δ-aminolevulinic acid synthetase activity,[15] the increase in δ-aminolevulinic acid synthetase activity in Harderian gland may not be correlated to heme.

Since the Harderian gland is an apocrine gland,[16] which is distinct histologically from the liver, an excess of porphyrin formed might be secreted into eyepit *via* the gland duct as the red tears. It could be considered that the chromodacryorrhea derived by pantothenic acid deficiency is an especial type of the porphyria, Harderian gland porphyria.

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13) In preparation.

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