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ENZYME HISTOCHEMISTRY OF THE SMALL INTESTINE IN INHERITED ICHTHYOSIS

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Abstracts. Enzyme histochemistry of biopsies from the small intestine of 5 patients with different forms of inherited ichthyosis and of 2 normal volunteers was performed. Two of the patients had ichthyosis vulgaris, two had non-bullous congenital ichthyosiform erythroderma and one had X-linked ichthyosis. The following enzymatic activities were examined: G6P-D, 6PG-D, $NADPH_2$-TR, ALD-A, L-D, C-A, IC-D, S-D, M-D, $NADH_2$-TR, ATP-AI, ATP-A II, ATP-A III, ATP-A IV, R5P-A, DHO-D, αGP-D, βHOB-D, MAO, GL-D, αGP-A I, αGP-A II, βGP-A II, N.EST-A. No significant variations in the different enzymatic activities were found for the ichthyosis vulgaris and non-bullous C.I.E. cases. More pronounced variations were found in X-linked ichthyosis, with a decrease in C-A, IC-D, R5P-A, βHOB-D, GL-D, αGP-A II and N.EST-A activity. Succinic dehydrogenase activity has been reported in the literature to be reduced in ichthyosis vulgaris and bullous C.I.E. However, the results obtained for our patients showed equal or higher reaction levels than in the controls.

Key words: Inherited ichthyosis; Small intestine; Enzyme histochemistry; Intestine in ichthyosis

Fry et al. (6) reported reduced enzymatic activity in the enterocytes of 50% of their ichthyosis vulgaris and bullous C.I.E. cases, and also some morphological alterations of the intestinal mucosa for some patients from the same group. Fry & McMinn(7) suggested that the histochemical succinic dehydrogenase test may be useful to detect metabolic activity alterations in the intestinal epithelial cells, especially as an aid in the diagnosis of idiopathic steatorrhea. Hooft et al. (12, 13) and Guilleminault et al. (11) described intestinal malabsorption in patients with Sjögren-Larsson syndrome, of which non-bullous C.I.E. is a part. On the basis of these observations some investigators suggested the possibility that both morphological and enzymatic enterocyte alterations may lead to a picture of intestinal malabsorption, which may be the principal pathogenic mechanism responsible for the development of ichthyosiform dermatoses.

The objective of this paper is to determine the existence of the alterations in the histoenzymological profile of epithelial cells from the small intestine of 5 inherited ichthyosis patients when compared with the normal state.

CLINICAL MATERIAL

A general physical and dermatological examination, a genealogical survey and histopathological skin examinations were carried out to diagnose and classify the types of ichthyosis affecting the patients. The criterion used for classification was that of Wells & Kerr (28), modified by Esterly (5). Five patients with inherited ichthyosis were studied: 2 with ichthyosis vulgaris (autosomal dominant), 2 with non-bullous C.I.E. (autosomal recessive), and 1 with X-linked ichthyosis (recessive). Biopsies taken from two volunteers under the same experimental conditions were used as controls.

METHOD

Intestinal biopsies were taken from the patients at the level of the duodenum–jejunum flexure (Treitz' corner) by means of an intestinal tube with a Crosby-type capsule. The position of the capsule was controlled radioscopically. The material obtained was immediately frozen by immersion in liquid nitrogen for 30 sec, and later cut into 10 μm thick sections with a CTD Harris cryotome (International Equipment Co., Needham Heights, Mass.). The sections were placed on 20×20 slides, and air dried prior to the histoenzymological studies.

The following enzymatic activites were studied: EEC 1.1.1.49 glucose 6-phosphate-dehydrogenase (G6P-D) (23); EC1.6.99.1 $NADPH_2$-tetrazolium reductase ($NADPH_2$-TR) (18); EC 4.1.2.13 F-1,6-P aldolase (ALD-A) (1); EC 1.1.1.27 lactate-dehydrogenase (L-D) (24); EC 4.2.1.3 cysaconitase (C-A) (29); EC 1.1.1.41 isocitrate-dehydrogenase (IC-D) (26); EC 1.3.99.1 succinate-dehydrogenase (S-D) (21); EC 1.1.1.37 malate-dehydrogenase (M-D) (24); EC 1.6.99.3 $NADH_2$-tetrazolium reductase ($NADH_2$-TR) (18); EC 3.6.1.3 adenosine-triphosphatase at pH 9.4 (ATP-A I), pH 8.5 (ATP-A II), pH 7.4 (ATP-AIII) and at pH 6.3 (ATP-A IV) (20); EC 3.1.3 ribose-5-phosphatase (R5P-A) (27); EC 1.3.3.1 dihydro-orotate-dehydrogenase (DHO-D) (2); EC 1.4.1.3

Table I. *Histoenzymology of the intestinal mucosa in 5 cases of inherited ichthyosis and in 2 controls. Intensity of reaction in the villi and cryptae*

	Villi						
	Ichthyosis vulgaris		Non-bullous C.I.E.		X-linked ichthyosis		
Enzyme	Case 1	Case 2	Case 3	Case 4	Case 5	Control 1	Control 2
G6P-D	+++++	+++++	*	*	*	+++++	+++
6PG-D	+++	+	*	*	*	++++	++
$NADPH_2$-TR	+++++	+	*	*	*	+++++	+++++
ALD-A	++	+	*	*	++	++	+
L-D	++	*	*	*	+++	+++	++
CA-D	+++	+++	+++	*	+	++++	+++
IC-D	++++	++++	*	*	++	+++++	+++++
S-D	+++	++	+++	+++	++	++	++
M-D	++	++++	++++	++++	*	++++	+++
$NADH_2$-TR	++++	+	*	*	++++	+++++	+++++
ATP-A I	+	+	+	+	+	++	+
ATP-A II	++++	+++++	+++++	++++	+++	+++++	++
ATP-A III	++	+++++	++++	+++++	++	+++++	++
ATP-A IV	*	+	*	+	+	++	+
R5P-A	++++	++++	*	*	++	+++++	++++
DHO-D	+	0	*	*	0	0	0
MAO	+++	*	*	*	*	++++	+++
GL-D	++	+	*	*	+	++	++
αGP-D	++	+	++	+	+	++	+
βHOB-D	++++	+++	+++	+++	+	++++	+++
αGP-A I	+++	++++	*	*	+++	+++++	++++
αGP-A II	++	++	++++	++++	+	+++	++

* = Unperformed reaction (see details in the text).

glutamate-dehydrogenase (GL-D) (4); EC 1.4.3.4 monoamino-oxidase (MAO) (8); EC 1.1.1.8 α-glycerophosphate-dehydrogenase (αGP-D) (25); EC 1.1.1.30 β-hydroxybutyrate-dehydrogenase (βHOB-D) (25); EC 3.1.3.1 alkaline phosphatase (αGP-A I) (9); EC 3.1.3.2 acid phosphate (αGP-A II) (10); EC 3.1.1. naphthol-acetate esterase (N.EST-A) (15). Histochemical reaction with Sudan Black, *ad modum* Wegmann & Fouquet (22), was carried out to evaluate the amount of lipids present in the enterocyte.

The following convention was used to indicate reaction intensity: 0, negative reaction; +, very weak reaction; ++, weak reaction; +++, moderate reaction; ++++, strong reaction; +++++, very strong reaction. The villi and cryptae were analysed separately.

RESULTS

The results obtained for the control and patient biopsies are summarized in Table I. Histochemical analysis revealed a sharp difference in intensity between the enzymatic activities of the enterocytes from the villi and cryptae. The outstanding enzymatic activities found for the enterocytes of the villi were those of the pentose cycle and of the respiratory cycle.

Comparison between biopsies from patients and from the controls was not always satisfactory due to the small amount of material collected from the former group, which did not allow a sufficient number of sections for all enzymes or did not contain villi and cryptae in the same fragment. This is why several reactions in Table I are marked as "unperformed".

In ichthyosis vulgaris (cases 1 and 2), the enzymatic profile of the enterocytes of the villi shows the same pattern as that in the controls. An adequate comparison of the cryptae was not possible. As to the individual enzymes, a few discrete variations in reaction intensity occurred in the patients' villi. In case 1, M-D, $NADH_2$-TR and alkaline phosphatase activity decreased, while S-D activity increased. In case 2, a decrease in $NADH_2$-TR, $NADPH_2$-TR, 6PG-D and GL-D activity was observed. However, these variations are very small, as shown in Table I.

In non-bullous C.I.E. (cases 3 and 4), there were variations in S-D activity only, which increased in relation to the controls, the variations being equal and of low intensity. In X-linked ichthyosis (case

Cryptae						
Ichthiosis vulgaris		Non-bullous C.I.E.		X-linked ichthyosis		
Case 1	Case 2	Case 3	Case 4	Case 5	Control 1	Control 2
*	*	*	*	*	+	0
*	*	*	*	*	0	0
*	*	*	*	++	+	++
*	0	*	*	+	+	0
*	*	*	*	+	*	0
*	*	*	*	*	++	++
+	*	*	*	*	++	++
*	*	*	*	*	+	0
*	++	*	++	*	++	+
*	*	*	*	++	++	++
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
*	0	*	0	0	0	0
*	*	*	*	0	++	+
0	0	*	*	0	0	0
*	*	*	*	*	++	+
*	*	*	*	0	0	0
*	*	*	+	*	+	0
*	*	+	+	*	++	++
*	*	*	*	+	+	0
*	*	*	*	0	++	0

5), variations were observed in C-A, IC-D, $NADH_2$-TR, R5P-A, βHOB-D, GL-D, αGP-A I, αGP-A II and N.EST-A, with a reduction in activity for all of them. The variations were more intense in the two lipid metabolism enzymes (βHOB-D and N.EST-A), the latter even being negative.

No decrease in reaction intensity was observed in the patients' villi for succinic dehydrogenase; on the contrary, in 3 cases (cases 1, 3 and 4) the reaction was slightly more intense than in the controls (see Fig. 1*a*, *b*, *c*).

The results for histoenzymological reactions in the cryptae region were compromised by the absence of such structures from most of the intestinal mucosa preparations.

DISCUSSION

The enzymatic profile observed in our preparations of normal intestinal mucosa can be compared with the profiles described by Dawson & Pryse-Davis (3) and Padykula et al. (16). The two sets of profiles are similar, but IC-D and $NADH_2$-TR, described by these authors as reactions of weak intensity, had strong ($NADH_2$-TR) or moderate to strong (IC-D) intensity in our experiments. These same authors described S-D as being of moderate intensity, whereas this enzymatic activity appeared to have little intensity in the controls in our study. βHOB-D, described as having strong intensity in the investigations quoted, showed moderate to strong intensity in our study. As to the other enzymes for which a comparison was possible, reaction intensity in the intestinal villi was similar.

Shonk et al. (19) studied the enzymatic profile of the human colon and rectum with biochemical techniques. A few of the enzymes studied by us are among those also studied by these investigators. Their observations showed the following sequence in decreasing order of enzymatic activity: MD/LD/ALD-A/αGP-D/G6P-D, while our sequence was G6P-D/M-D/L-D/ALD-A/αGP-D. The only marked discrepancy between the two studies concerns G6P-D activity, which may be due to functional differences between the small and large bowel.

As can be seen in Table I, S-D enzymatic activity in the ichthyosis cases was identical with (cases 2 and 5) or more intense (cases 1, 3 and 4)

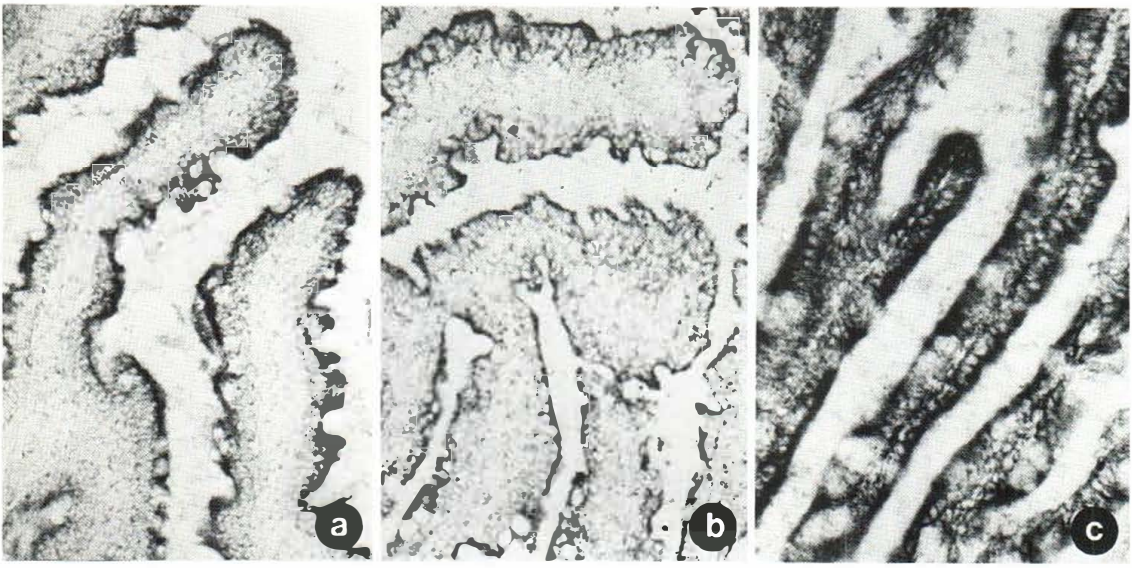

Fig. 1. Histoenzymology in intestinal villi. Succinate-dehydrogenase in (*a*) normal control; (*b*) X-linked ichthyosis, and (*c*) ichthyosis vulgaris. Original magnification, ×50.

than in the controls. Fry et al. (6) described these enzymes as decreased in 50% of their ichthyosis cases, and Fry & McMinn (7) had previously suggested that a decrease in the activity of this enzyme may be indicative of idiopathic steatorrea. This finding would strengthen the hypothesis that there is an association between intestinal malabsorption and inherited ichthyosis. It was not possible to confirm the results of Fry et al. (6) in our study. It is important to point out that these authors did not mention the criteria used for normality in their investigation, which makes it difficult to compare their data with ours; Dawson & Pryse-Davies (3) and Padykula et al. (16) considered the level of S-D enzymatic activity in the normal jejunum of man as moderate, and, as can be interpreted from their texts, their S-D results seem to be the same as ours.

We noted a reduction in activity for several enzymes (6PG-D; $NADPH_2$-TR; CA-D; IC-D; M-D; $NADH_2$-TR; R5P-A; GL-D; βHOB-D; αGP-A I; αGP-A II) (Table I), but the values varied for the different forms of ichthyosis and for the different patients within the same type of ichthyosis, so that, except for the fact that a depression in activity was a constant finding, it was not possible to isolate any enzyme which would discriminate any particular form of ichthyosis.

Monges et al. (14) observed an increase in $NADH_2$-TR, $NADPH_2$-TR and G6P-D activity and a reduction in L-D activity during lipid absorption. In our study, there was no variation between L-D and G6P-D activity; there was a marked reduction in $NADH_2$-TR and $NADPH_2$-TR activity only in case 2 (ichthyosis vulgaris), with no variations observed in the other patients. It is difficult to attribute a pathological significance to this finding. As to the variations found for the other enzymes studied in the cases of ichthyosis vulgaris and non-bullous C.I.E., these must be individual variations due to the reduced number of enzymes involved and to the slight intensity of such variation.

In relation to the intense variations observed in case 5 (X-linked ichthyosis), involving a large number of enzymes, it is more difficult to give emphasis to this finding in view of the good general clinical condition of the patient and of the normal results of absorption tests performed, as per Pina Neto et al. (17). To further clarify these data it would be necessary to increase the number of cases under study.

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