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Determination of Histidine α-Deaminase in Human Stratum Corneum and its Absence in Histidinaemia

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In studies on the nature of the enzymic defect in patients with histidinaemia, an inborn error of histidine metabolism (La Du, Howell, Jacoby, Seegmiller & Zannoni, 1962), it was necessary to develop a sensitive assay procedure to measure histidine α-deaminase (L-histidine ammonia-lyase, EC 4.3.1.3), the enzyme that converts histidine into urocanic acid. The application of this method has shown that the stratum corneum of normal human skin is a rich source of histidine α-deaminase. However, this layer in two patients (sibs) with histidinaemia did not contain any detectable histidine α-deaminase, and the lack of this enzyme appears to be the biochemical defect in histidinaemia.

EXPERIMENTAL

Reagents. L-Histidine monohydrochloride monohydrate (Mann Research Laboratories, New York, U.S.A.) (105 mg.) in water was neutralized with 1 N-NaOH and made up to 5·0 ml. with water. Glutathione (reduced) (Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.) (305 mg.) was dissolved in 10 ml. of 0·1 M-sodium pyrophosphate buffer, pH 9·2. Urocanic acid dihydrate (Mann Research Laboratories) (8·7 mg.), having ϵ 18 800 at 277 mμ at pH 7·5, was dissolved in 50 ml. of mN-NaOH.

Preparation of guinea-pig tissue homogenates. The abdominal area of the guinea pigs was shaved before the animals were decapitated. Immediately thereafter, various tissues were excised and a 10% (w/v) homogenate was prepared in 0·01 M-sodium pyrophosphate buffer, pH 9·2. Samples of skin were dissected into epidermal, dermal and subcutaneous layers. All tissues were minced before homogenization in a Waring Blendor for 1 min. The crude homogenates were spun at 3000*g* for 10 min. and the supernatant fraction was used for the assays of histidine α-deaminase and urocanase.

Preparation of homogenates of human stratum corneum. Samples of stratum corneum (25–50 mg.) were removed with nail clippers from the thick cornified skin adjacent to the finger-nails. Homogenates (2%, w/v) were prepared by grinding the tissue with sufficient 0·01 M-sodium pyrophosphate buffer, pH 9·2, in a glass-walled TenBroeck-type tissue grinder (7 ml. capacity).

Portions (1 ml.) of the homogenates were each dialysed against 1 l. of 5 mM-sodium pyrophosphate buffer, pH 9·2, containing 0·1 ml. of 0·1 M-glutathione, for 1 hr. to remove urocanic acid. Under these conditions over 90% of the urocanic acid was removed.

Assays of histidine α-deaminase and urocanase. For homogenates of guinea-pig tissues, the method described by Tabor & Mehler (1955) was used. The assay for histidine α-deaminase is based on the accumulation of urocanic acid at 277 mμ at pH 9·2. Urocanase does not interfere with the assay for histidine α-deaminase at this pH. Urocanase activity was measured spectrophotometrically at 277 mμ by observing the rate of disappearance of urocanic acid at pH 7·5.

Because of the turbidity of homogenates of human stratum corneum, the direct spectrophotometric assay could not be used. Control and experimental tubes were incubated with 0·50 ml. of 0·1 M-sodium pyrophosphate buffer, pH 9·2, and 0·05 ml. of 0·1 M-glutathione (neutralized). The experimental tubes contained 0·05 ml. of 0·1 M-L-histidine (neutralized) and the control tubes contained 0·05 ml. of 0·1 M-sodium pyrophosphate buffer, pH 9·2. Homogenate (0·4 ml.) of human stratum corneum was added to each of the control and experimental tubes and the total volumes were adjusted to 1·2 ml. with water. The control tubes were incubated at 37° in duplicate and the experimental tubes in triplicate for each assay. The samples (0·2 ml.) were removed at zero time and after 2 hr. or, in some experiments, more frequently. The samples were immediately deproteinized in 0·4 ml. of 7% (w/v) perchloric acid and kept in an ice bath for 10 min. The acidified samples were then spun at 3000*g* for 10 min. and the extinctions of the supernatant fractions read at 277 mμ in a Beckman DU spectrophotometer. One μg. of urocanic acid gave a reading of 0·260. Under these conditions, it was possible to detect the formation of less than 0·25 μg. of urocanic acid/hr. with accuracy.

Determination of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The protein concentration of the 2% (w/v) stratum-corneum homogenates averaged 9 mg. wet wt./ml. and did not vary significantly from one preparation to another.

RESULTS AND DISCUSSION

Distribution of histidine α-deaminase in guinea-pig tissues. The histidine-α-deaminase activity in homogenates of various guinea-pig tissues was determined by the spectrophotometric assay method of Tabor & Mehler (1955). (Table 1). Relatively high activity was found only in liver and the epidermal layer of the skin, in agreement with the report of Schwarz (1961). Neither the dermal nor subcutaneous layers contained any detectable amounts of the enzyme and, in contrast with the liver, the

epidermal layer of skin contained no detectable urocanase activity. The rate of urocanic acid formation was linear with time in the presence of the epidermis homogenate. Urocanic acid incubated with homogenates of whole skin was comcompletely stable at either pH 7·5 (the optimum pH for liver urocanase activity) or at pH 9·2 (within the optimum pH range for histidine-α-deaminase activity).

Histidine α-deaminase in human stratum corneum. The rate of formation of urocanic acid in preparations of stratum corneum was linear with time for at least 2 hr., and was proportional to the concentration of homogenate employed. No urocanic acid accumulated in the presence of boiled homogenate, or if the homogenate was omitted from the incubation tube. Urocanic acid incubated under identical conditions (both at pH 7·5 and pH 9·2) remained unchanged; this indicates that the homogenates contain no urocanase.

Histidine α-deaminase in the stratum corneum of normal and histidinaemic individuals. Homogenates were prepared of stratum corneum from members of a family with histidinaemia and from a group of normal children and adults. The children with histidinaemia had no detectable histidine α-deaminase in their skin when assayed on three occasions (Table 2). Their unaffected sister had histidine-α-deaminase activity considerably below the average range encountered in normal children. This finding suggests that she may be a carrier of the metabolic defect. The mother's histidine-α-deaminase activity was low but within the normal range, and the father's was below the normal range. The sweat of the two affected children contained no urocanic acid (La Du *et al.* 1962), but that from the parents, unaffected sister and normal individuals contained urocanic acid.

Other biochemical findings in the histidinaemic sibs, such as elevated blood histidine concentrations, the excretion of histidine and imidazolylpyruvic acid, and the ability to metabolize intravenously administered urocanic acid to form formiminoglutamic acid (La Du *et al.* 1962), leave no doubt that the defect in these children is similar to the cases reported by Ghadimi, Partington & Hunter (1961, 1962) and by Auerbach, DiGeorge, Baldridge, Tourtellotte & Brigham (1961, 1962).

It was suggested by Ghadimi *et al.* (1961, 1962) that this metabolic disease was probably a defect in histidine metabolism. Auerbach *et al.* (1961, 1962) identified imidazolylpyruvic acid in urine and presented additional evidence that the enzymic defect was most likely to be a deficiency of 'histidase' (histidine α-deaminase). This is confirmed by the results given in Table 2.

The function of histidine α-deaminase in the stratum corneum is not known. Zenisek, Kral & Hais (1955) and Hais & Zenisek (1959) have proposed that the urocanic acid protects the skin from ultraviolet radiation. However, the clinical history

Table 1. *Histidine α-deaminase in guinea-pig tissues*

Experimental details are given in the text. The results are given as means with the ranges in parentheses. Lung, adrenal, brain and plasma had no detectable activity.

Tissue	No. of animals	Histidine-α-deaminase activity (μmoles of urocanic acid formed/hr./g. wet wt.)
Liver	5	1·40 (1·37–1·43)
Skin	4	0·17 (0·15–0·18)
Epidermis	4	0·49 (0·48–0·51)
Dermis	4	0·01 (<0·01–0·01)
Subcutaneous	4	0·01 (<0·01–0·01)
Heart	2	0·09 (0·08–0·09)
Spleen	2	0·05 (0·04–0·05)
Testes	2	0·03 (0·03)
Intestine	2	0·03 (0·02–0·03)
Kidney	2	0·01 (<0·01–0·01)
Muscle	2	0·01 (<0·01–0·01)

Table 2. *Histidine α-deaminase in the stratum corneum of members of a family with histidinaemia and of normal individuals*

Experimental details are given in the text. The results are given as means with the ranges in parentheses.

	Age (years)	No. of determinations	Histidine-α-deaminase activity in stratum corneum (μmoles of urocanic acid formed/hr./g. wet wt.)
Histidinaemic family			
Case 1, female	6	4	0 (<0·01)
Case 2, male	5	3	0 (<0·01)
Sister	4	3	2·0 (1·91–2·14)
Mother	25	3	2·3 (2·21–2·43)
Father	31	3	1·4 (1·20–1·59)
Normal controls			
Children	—	8	5·4 (4·2–6·6)
Adults			
Female	—	12	3·0 (1·8–5·3)
Male	—	12	3·6 (1·9–5·3)

of these children failed to reveal any evidence of unusual sensitivity of their skin to sunlight or drugs, or any occurrence of skin rashes or allergic manifestations. The skin of the affected children showed no defect in keratinization and under ultraviolet light it did not differ from that of normal children. The only clinical finding of significance was that both these histidinaemic children had a speech defect (La Du *et al.* 1962). One of the two affected sibs reported by Ghadimi *et al.* (1962) and the histidinaemic child studied by Auerbach *et al.* (1962) also had defects in speech.

SUMMARY

1. A spectrophotometric method to measure histidine α-deaminase in the stratum corneum of normal human skin is described.

2. Normal human stratum corneum lacks urocanase.

3. The skin of two sibs with histidinaemia does not contain any detectable histidine α-deaminase.

4. This lack of histidine α-deaminase would appear to be the biochemical abnormality responsible for histidinaemia.

5. The distribution of histidine α-deaminase in several tissues of the guinea pig has also been examined.

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The Distribution of Peptidases in Subcellular Fractions from the Mucosa of the Small Intestine of the Rat

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The studies of Newey & Smyth (1959, 1960) established that dipeptides entered the intestinal mucosa and were hydrolysed to free amino acids within the mucosa. G. B. Robinson (unpublished work) subsequently obtained evidence that intestinal peptidases were located primarily within the epithelial cells. Thus hydrolysis of absorbed dipeptides presumably occurs during transfer of the dipeptides across the epithelial cells. The intracellular distribution of intestinal peptidases has now been investigated to obtain information about the site of peptide hydrolysis within the epithelial cells.

A number of workers have reported difficulties in the isolation of subcellular components from small-intestinal mucosa. Schmidt, Bessman & Tannhauser (1957) found that mucus, released from the mucosal cells on homogenization, interfered with the fractionation procedure. Baker (1958) and Busch, Davis & Anderson (1958) also reported that mucus interfered with fractionation. To overcome this problem, different homogenizing media have been used. Schmidt *et al.* (1957) used an iso-osmotic salt solution and Buell & Reiser (1959) used 0·6% sodium chloride. Epstein & Shapiro (1959) and Senior & Isselbacher (1960) used iso-osmotic potassium chloride; the latter authors later reported the use of iso-osmotic mannitol (Senior & Isselbacher (1961). Hübscher, Clark & Webb (1962) have used barium sulphate to remove mucus, and Glover & Green (1957) described a technique for washing the particulate components from the adhering mucus. Allard, de Lamirande & Cantero (1957), Triantaphyllopolous & Tuba (1959) and Borgström & Dahlquist (1958) have reported

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