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QUANTITATIVE HISTOCHEMISTRY OF THE PRIMATE SKIN

VI. Lactate Dehydrogenase*

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Lactate dehydrogenase (LDH)[1] catalyzes the final step in the Embden-Meyerhof glycolytic pathway, *i.e.*, the reduction of ketopyruvic acid to lactic acid, utilizing the hydrogen of NADH[1].

The presence of this enzyme in skin was suggested as early as 1926 by Wohlgemuth and Klopstock who demonstrated that surviving skin synthesizes lactic acid from glucose (1). Recent isotopic studies show that 40 to 70% of labelled glucose is converted to lactic acid in human and rat skin (2, 3). A direct measurement of LDH activity in skin was attempted in 1955 by Hershey and Mendle (4), and since then a number of workers have measured LDH activities in normal and pathologic human skin (5, 6) and in rat skin (7).

The basic nature and characteristics of this enzyme, however, do not appear to have been studied in detail. In this paper we present the results of a study of the quantitative distribution of LDH in the skin of the rhesus monkey and stump-tail macaque, and some kinetic characterization of this enzyme in the epidermis of the rhesus monkey.

MATERIAL AND METHODS

A method for preparing epidermal homogenates from the skin of the rhesus monkey has already been described (8). LDH activity was measured in the direction of glycolysis, *i.e.*, pyruvate to lactate. The coupled NAD^+ formation was measured with a fluorometric procedure as described previously (9), except for the assay reagent mixture which consisted of 1 mM sodium pyruvate[2]; 2 mM NADH; 0.05% bovine plasma albumin; 0.1 M Tris buffer, pH 7.6; and 5 μl of epidermal homogenate (1%) in a total volume of 105 μl. The reverse reaction was also tested with lactate as substrate as described below (see *Results*).

For the quantitative histochemical study of LDH activity, 0.5 to 5.0 μg samples were dissected out from frozen-dried skin and placed into micro test tubes. Subsequently, 18 μl of the reaction mixture was added to each sample and incubated for 60 minutes at 37° C. Five μl of 1 N HCl were added to stop the reaction, and 15 μl aliquots of the mixture were transferred into 3 ml fluorometer tubes for the measurement of the resulting NAD^+ as described above. Standard tubes containing 5 to 20 mμmoles NAD^+/tube and blank tubes without tissues were run simultaneously.

Lactic acid was measured according to the method of Barker and Summerson (10).

RESULTS

The effects of various pH's on the LDH activity are shown in Fig. 1. The optimal pH is 7.5. LDH decreases its activity markedly in alkaline pH. Increased pyruvate concentration resulted in a remarkable substrate inhibition (50% inhibition at 8 mM) (Fig. 2). The Michaelis constant for pyruvate was 3×10^{-4} M at pH 7.6, Tris buffer with NADH concentration of 2 mM. The optimum concentration of NADH was 2 mM (Fig. 3). It appears that NADH concentration higher than 4 mM caused slight inhibition of the LDH activity. The Km for NADH with epidermal homogenate as an enzyme source was approximately 4×10^{-4} M at pH 7.6 with 1 mM of pyruvate concentration. In Fig. 4, linear rates of enzyme reaction were obtained over a wide range, nearly up to 85 mμmoles NAD^+ production per tube.

The stoichiometry of the reaction catalyzed by epidermal LDH was tested in the same reagent mixture except that the enzyme concentration was increased 20-fold. Lactic acid formation was measured colorimetrically (10). The disappearance of NADH (1.20 μmoles/ml) corresponded well with the formation of both NAD^+ (1.10 μmoles/ml) and lactic acid (1.28 μmoles/ml).

The reverse reaction with lactate as a substrate was active in alkaline ranges. The initial velocities at pH 7.2 and 9.5 were 0.2 moles and

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[1] Abbreviations used are LDH = lactate dehydrogenase; NADH and NAD^+ = nicotinamide adenine dinucleotide, reduced and oxidized forms.

[2] All chemicals were commercially available and of reagent grade.

1 mole/kg wet weight/hr in a test system consisting of 250 mM lithium lactate and 1 mM NAD^+. However, practically no reaction was measured at 1 mM of lactate level in this system at pH 7.2. The equilibrium of LDH reaction at physiological pH appears to be to the direction of glycolysis.

Table I shows the distribution of lactic dehydrogenase in the different structures of the skin. Whereas, in general, this enzyme was twice as active as glyceraldehyde-3-phosphate dehydrogenase (11), both enzymes were distributed in similar proportions in the skin and its appendages. The considerable amount of LDH activity in the keratin layer and the relatively lower values in the sebaceous glands as compared to other parts coincide with the

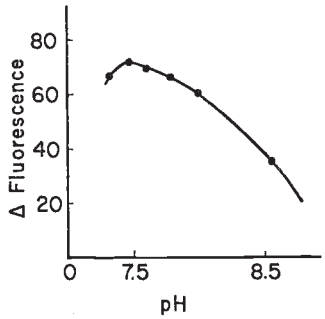

FIG. 1. pH activity curve of the epidermal enzyme in 0.1 M Tris buffer system. The assay method as described in text. The ordinate is enzyme activity expressed as the fluorescence, which is the microammeter reading.

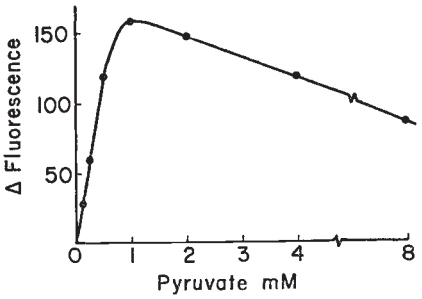

FIG. 2. Effect of pyruvate concentration. The test system as in Fig. 1 at pH 7.6, 0.1 M Tris buffer.

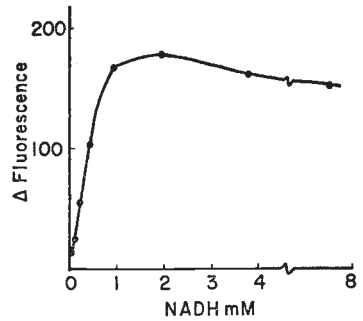

FIG. 3. Effect of NADH concentration. The assay system as in the text with different levels of NAD^+.

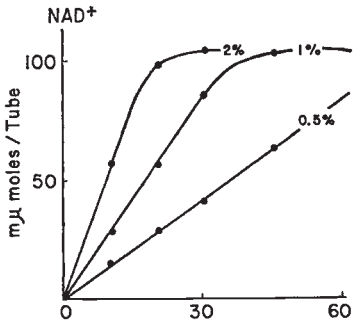

FIG. 4. Time course and effect of enzyme concentration. The assay system as described in the text. The concentration of the epidermal homogenate (%) used is indicated in this figure.

TABLE I

*Lactic dehydrogenase activity in various skin structures**

Structure	Rhesus monkey	Stump-tail macaque
SCALP		
Epidermis	14.5	16.5
Hair follicle	13.2	23.4
Eccrine gland	21.4	16.6
Apocrine gland	—	24.6
Sebaceous gland	5.2	8.7
Dermis	2.4	0.91
SOLE		
Keratin layer	6.0	2.1
Granular layer	9.1	10.4
Prickle layer	12.6	14.4
Basal layer	17.2	15.6
Sweat gland	15.2	18.2
Dermis	0.5	1.4
LIP		
Mucous membrane, upper	7.1	15.4
Mucous membrane, lower	8.8	19.2
Sebaceous gland	9.7	15.2

* Enzyme activity is expressed as moles of pyruvate converted to lactate per kilogram of dry weight tissue per hour (moles/kg dry wt/hr). Each figure is the mean of 5 to 9 determinations.

data for glyceraldehyde-3-phosphate dehydrogenase.

DISCUSSION

The properties of LDH in the skin of primates generally appeared to coincide with those in other mammalian tissues (12, 13); the topographical distributions of this enzyme are also similar to the data on human skin with the exception of the hair follicles (5). The moderate LDH activity (2–6 moles/kg dry wt/hr) found in the keratin layer is probably due to the high stability of this enzyme structure, as in the case of glyceraldehyde-3-phosphate dehydrogenase (11).

It is noteworthy that the distribution pattern of LDH in primate skin coincides with that of glyceraldehyde-3-phosphate dehydrogenase. The latter enzyme requires NAD^+ for the oxidation of glyceraldehyde to glyceric acid and reduces it to NADH, which subsequently will be utilized by LDH. The NADH-coupled production of lactic acid is, therefore, essentially a mechanism for the regeneration of NAD^+ which then participates again as a hydrogen acceptor in the oxidation of glyceraldehyde.

SUMMARY

Lactate dehydrogenase is one of the most active enzymes in the skin of primates. In the skin of rhesus and stump-tail macaques it is distributed in similar quantity (13.2 to 24.6 moles/kg dry wt/hr) through the epidermis, hair follicles and sweat glands. As compared with the above three regions, the sebaceous glands have relatively lower LDH activity (5.2–8.7 moles/kg dry wt/hr). In the epidermis of the sole, the basal layer has more enzyme activities (15.6–17.2 moles/kg dry wt/hr) than the upper layers (9.1–14.4 moles/kg dry wt/hr) and the keratin layer has considerable enzyme activities (2.1–6.0 moles/kg dry wt/hr). The properties of lactate dehydrogenase in the skin appear to be similar to those in mammalian tissues hitherto reported.

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