

ADENYLATE KINASE, ATP PHOSPHOHYDROLASE AND
GLYCERALDEHYDEPHOSPHATE DEHYDROGENASE
ACTIVITIES IN THE NORMAL HUMAN SKIN AND IN SOME
PAPULOSQUAMOUS DISEASES OF THE SKIN

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Abstract. The activity of adenylate kinase (AK), ATP phosphohydrolase (ATPase) and glyceraldehydephosphate dehydrogenase (GAPDH) was measured in various epidermal layers in skin from healthy controls and from patients with psoriasis, neurodermatitis, lichen planus and pityriasis rosea. The assays were performed on microdissected specimens prepared from cryostat sections according to Lowry's microtechniques. AK activity was increased about 60% in the psoriatic lesion and differed in this respect from the affected skin in neurodermatitis, lichen planus and pityriasis rosea in which the increases in AK activity were less pronounced. The enzyme displayed higher activities in the basal than in the subcorneal epidermis. The ATPase activity did not differ between controls and psoriatic patients. The GAPDH activity was increased by various degrees in all four lesions studied. It rose about 90% in epidermal layers of the psoriatic lesion and about 90% in subcorneal and 60% in basal epidermis of the lichen planus lesion. The relative epidermal height in psoriasis, neurodermatitis and lichen planus showed no correlation with enzymatic activities of AK and GAPDH.

In an accompanying paper (7) a report is given on the ATP¹ and ADP contents of normal epidermis and of epidermal material obtained from patients with psoriasis, neurodermatitis and lichen planus. The general view that the adenylate pool in a specified tissue is fairly constant (9) can be applied to the results obtained from healthy skin, neurodermatitis and lichen planus (7). The pool seems to be increased, however, in the psoriatic lesion (7).

ATP is a necessary co-factor in many energy-requiring reactions such as the synthesis of proteins and nucleotides (9). The interconversion

rate between the adenylates is of importance in this context. The reaction,



is catalysed by the enzyme AK (11). This enzyme serves as a regulator between ATP and ADP when AMP is accumulating during biosynthesis (9). The enzymatic activity of AK was therefore measured on the same material as the epidermal adenylates described in the accompanying paper (7). An assay of ATPase was included in order to investigate the turnover of ATP to ADP in the control and psoriatic materials.

The formation of ATP by anaerobic metabolism is achieved in glycolysis by the phosphoglycerate and the pyruvate kinase steps (9). Closely related to the first of these steps is that of GAPDH. The activities of both GAPDH and pyruvate kinase were increased in psoriasis (8). In the present study the analysis of the GAPDH activity was extended to include neurodermatitis, lichen planus and pityriasis rosea in order to compare enzymatic activity between psoriasis and the other three dermatoses which, in different ways, show similarities to psoriasis (6).

MATERIAL AND METHODS

Punch biopsies were collected without anaesthesia from patients with psoriasis, neurodermatitis, lichen planus and pityriasis rosea and from healthy controls. The number of patients in the different groups and the length of their illness are summarized in Table I. The lesions were guttate or papular and the criteria for their diagnosis were the same as described earlier (6). The biopsies were taken from the extensor region of the forearm, one from the

¹ For abbreviations, see legend to Table II.

Table I. Composition of the patient material

Relative epidermal height was measured as the ratio of the height of the subcorneal epithelium in an epidermal papilla to that over the adjacent corial papilla. Means and S.E.M. are given. Since measurements of relative epidermal height were made in only a small number of patients with pityriasis rosea none of the present patients were included. Means for 6 other patients are therefore included within parentheses; the values marked with an asterisk indicate the trunk as biopsy site

| Groups | No. | Age of patients | | Duration of illness | | No. | Epidermal height | |
|------------------|-----|-----------------|------|---------------------|----------|-----|------------------|--------------------------|
| | | Range | Mean | Range | Mean | | Non-involved | Involved |
| Controls | 20 | 20-47 | 26 | — | — | 19 | 2.18 ± 0.15 | — |
| Psoriasis | 24 | 11-61 | 33 | 0.1-35 | 15 years | 15 | 1.80 ± 0.12 | 4.08 ± 0.45 |
| Neurodermatitis | 8 | 25-71 | 56 | 2-12 | 4 years | 7 | 1.65 ± 0.06 | 4.62 ± 0.98 ^a |
| Lichen planus | 11 | 19-70 | 42 | 0.5-16 | 4 months | 4 | 1.75 ± 0.12 | 2.35 ± 0.13 ^a |
| Pityriasis rosea | 8 | 17-38 | 24 | 4-14 | 10 days | 6 | (2.12) (2.17*) | (2.94*) |

^a One value lost.

centre of a lesion and another from unaffected skin 40 mm away from the lesion in all cases except for those with pityriasis rosea. In these patients the biopsies were taken from a guttate lesion on the trunk and from non-involved skin 40 mm away from the lesion together with one from unaffected skin on the forearm. None of the patients had been treated during the month prior to biopsy and except for the present disease they considered themselves healthy.

The biopsy material was immediately frozen in cold isopentane (-86°C) and kept dry at this temperature in a Dewar vessel until sectioned. The sectioning was performed within 2 days and the further treatment of the material such as dissection and weighing was done as described earlier (5, 8). The dissected material comprised tissue from the basal part of the rete ridges and from the adjacent subcorneal epithelium.

Assay conditions. Chemicals were of reagent grade. Enzymatic preparations were purchased from Boehringer Mannheim GmbH, Germany.

Adenylate kinase. The analytical procedure (2) is summarized in Table II. Analyses of various tissues displayed a broad pH curve with an optimum near 8 (2, 11). Tests on pH dependence were therefore not made in the epidermal material. In preliminary tests the consumption of the substrate was proportional to the sample weight which ranged between 140 and 850 ng. Specimens in quadruplicate weighing about 300 ng were used. ATP and glucose 6-phosphate were run as standards. The coefficient of variation calculated on quadruplicates was 17%.

ATP phosphohydrolase. ATPase was measured (2) on quadruplicates by converting its reaction product ADP by pyruvate kinase and phosphoenolpyruvate as summarized in Table II. Sample weights of about 300 ng were used. The coefficient of variation calculated on quadruplicates was 33%.

Glyceraldehydephosphate dehydrogenase. GAPDH was measured on quintuplicates after capture of the primary reaction product by arsenate (15) as summarized in Table II. The pH dependence of the reaction in the range 7.0 to 9.5 displayed an optimal pH at 8.6. An initial study showed that the consumption of the substrate was propor-

tional to sample weights and incubation time in the ranges 240 to 600 ng and 20 to 60 min, respectively. Sample weights of about 450 ng were used in the final procedure. The coefficient of variation calculated on quintuplicates was 10%.

The activities of the enzymes were finally measured on a Farrand A fluorometer utilizing the appropriate nicotinamide adenine dinucleotide according to Lowry (10), as shown in Table II. The results are expressed as moles substrate converted per kg dry weight and hour of incubation (MKH).

Measurements of relative epidermal height were made as described in the preceding paper (7).

The statistical treatment consisted of *t*-tests or analyses of variance according to Snedecor (13) and Seeger (12).

RESULTS

The enzymatic activities obtained for the various diseases studied are summarized in Table III.

Adenylate kinase. The activities of AK found in controls were 3.9 and 5.5 MKH in subcorneal and basal epidermis, respectively. This difference was significant ($P < 0.01$). Similar activities were found in the non-involved skin taken from patients with the various diseases examined (Fig. 1). The difference between layers was significant as shown by analysis of variance ($P < 0.001$). AK activity was increased 30 to 90% in the various lesions. Except for pityriasis rosea, the lesions displayed significantly higher enzymatic activities in the basal than in the subcorneal epidermis ($P < 0.005$). The psoriatic lesion showed the highest activity of AK, which was significantly different from that in the other three diseases ($P < 0.001$). Similar enzymatic activities were found in neurodermatitis, lichen planus and pityriasis rosea (Fig.

Table II. Conditions for the assays

| Enzyme measured | Buffer | Substrate | Other additives | Incubation volume (μ l) | Time of incubation at 38°C (min) | Final volume (ml) | Product measured |
|-----------------|---|--|---|------------------------------|----------------------------------|-------------------|------------------|
| AK | 25 mM Tri-ethanolamine pH 7.6 | 1.9 mM ADP | 1.3 mM Glucose 1.0 mM NADP 0.6 mM EDTA 32 mM MgCl ₂ 0.7 U/ml HK 0.3 U/ml G6PDH 0.03% BPA | 10.4 | 60 | 0.13 | NADPH |
| ATPase | 42 mM Tri-ethanolamine pH 7.6 | 2.0 mM ATP | 1.2 mM Phospho- enolpyruvate 1.2 mM NADH 12 mM MgCl ₂ 135 mM KCl 0.5 U/ml PK 2.9 U/ml LDH 0.05% BPA | 9.7 | 60 | 0.13 | NAD |
| GAPDH | 25 mM Tris- (hydroxymethyl)- aminomethane pH 8.6 | 0.3 mM Gly- ceraldehyde- phosphate | 4 mM Disodium- arsenate 0.25 mM NAD 1.5 mM EDTA 5 mM Mercapto- ethanol 0.02% BPA | 4.17 | 60 | 0.17 | NADH |

ADP = Adenosine 5'-diphosphate
 AK = Adenylate kinase, EC 2.7.4.3
 ATP = Adenosine 5'-triphosphate
 ATPase = ATPphosphohydrolase, EC 3.6.1.3
 BPA = Bovine plasma albumin
 EDTA = Ethylenediamine tetraacetic acid
 GAPDH = Glyceraldehydephosphate dehydrogenase,
 EC 1.2.1.12

G6PDH = Glucose 6-phosphate dehydrogenase EC 1.1.1.49
 HK = Hexokinase, EC 2.7.1.1.
 LDH = Lactate dehydrogenase, EC 1.1.1.27
 NAD, NADH = Nicotinamide adenine dinucleotide, oxidized
 and reduced form
 NADP, NADPH = NAD-, NADH-phosphate
 PK = Pyruvate kinase, EC 2.7.1.40

1). These differed significantly from controls ($P < 0.001$).

ATP phosphohydrolase. These activities were measured in controls and in psoriatic patients (Table III). No differences were evident between the various groups examined. Means of the activities varied between 1.3 and 2.0 MKH.

Glyceraldehydephosphate dehydrogenase displayed higher activities in all of the lesion types studied, as compared with uninvolved skin ($P < 0.01$); Fig. 2. The activities in psoriasis increased 97% in subcorneal and 81% in the basal part of the epidermis and in the lichen planus lesion 91% and 64%, respectively. The increase in activity of GAPDH varied between 60 and 40% in neurodermatitis and pityriasis rosea. The difference between layers was significant only in the lesion of lichen planus ($P < 0.05$). Psoriasis and lichen planus were probably different from each other ($P < 0.05$) and both were different from the other two lesions ($P < 0.05 - 0.001$).

The relative epidermal height is summarized in Table I. These values showed no correlation to the enzymatic activity of AK in controls or psoriatic patients. No correlation was found between these values and GAPDH activity in controls or in patients with psoriasis, neurodermatitis or lichen planus.

DISCUSSION

The activity of AK (3.9 to 5.5 MKH) was relatively higher than some glycolytic enzymes recently measured (8). The increase of about 60% found in the affected psoriatic epidermis as compared with non-involved skin in these patients was also in line with the alterations shown for many other enzymes in this disease (4, 8). The sum of the ATP and ADP contents ranged between 10 and 15 mmoles/kg dry weight in various layers of epidermis in healthy skin as well as in psoriatic, neurodermite and lichen planus skin (7).

Table III. The activity of adenylate kinase, ATP phosphohydrolase and glyceraldehydrophosphate dehydrogenase in human epidermis

Means and S.E.M. are given. Values are expressed as moles of substrate consumed per kg dry weight and hour of incubation at 38°C (MKH)

| Group | No. | Non-involved epidermis | | Involved epidermis | |
|---|-----|------------------------|------------------------|------------------------|------------------------|
| | | Subcorneal | Basal | Subcorneal | Basal |
| <i>Adenylate kinase</i> | | | | | |
| Controls | 11 | 3.9±0.4 | 5.5±0.5 | — | — |
| Psoriasis | 11 | 4.0±0.3 | 4.9±0.3 | 6.6±0.5 | 8.0±0.4 |
| Neurodermatitis | 7 | 3.8±0.6 | 4.9±0.3 | 5.2±0.4 | 6.9±0.3 |
| Lichen planus | 8 | 3.5±0.2 | 4.9±0.3 | 5.2±0.5 | 6.5±0.3 |
| Pityriasis rosea | 6 | 3.6±0.5 | 4.7±0.2 | 6.0±0.4 ^a | 6.0±0.6 ^a |
| | 5 | 3.2±0.4 ^a | 4.2±0.5 ^a | | |
| <i>ATP phosphohydrolase</i> | | | | | |
| Controls | 8 | 1.3±0.1 | 1.4±0.2 | — | — |
| Psoriasis | 9 | 1.4±0.2 | 1.6±0.3 | 2.0±0.5 | 1.9±0.4 |
| <i>Glyceraldehydrophosphate dehydrogenase</i> | | | | | |
| Controls | 7 | 0.19±0.04 | 0.19±0.04 | — | — |
| Psoriasis | 11 | 0.24±0.02 | 0.26±0.03 | 0.47±0.04 | 0.46±0.03 |
| Neurodermatitis | 7 | 0.15±0.03 | 0.17±0.02 | 0.25±0.06 | 0.26±0.05 |
| Lichen planus | 10 | 0.22±0.02 | 0.22±0.03 | 0.42±0.02 | 0.36±0.02 |
| Pityriasis rosea | 6 | 0.18±0.04 | 0.17±0.03 | 0.31±0.05 ^a | 0.31±0.06 ^a |
| | 5 | 0.22±0.04 ^a | 0.20±0.04 ^a | | |

^a Biopsies taken from the trunk.

The capacity of AK to convert the epidermal adenylate content is thus at least 400 times this amount per hour. It is therefore probable that the AK reaction is near equilibrium and that the enzyme can hold the relative concentrations of adenylates within narrow limits. The AMP con-

centration was calculated on this basis in the preceding paper (7) in order to estimate the energy charge of the adenylate system according to Atkinson (1). A point which might still affect the estimate of the energy charge is whether AK is unevenly distributed within the various compartments of the cell. As indicated by Chappell (3), AK activity was found both in the mitochondria and in the cytosol of liver cells. This compartmentation of the enzyme would seem to constitute sufficient evidence against this objection. Another factor is the epidermal ATPase activity which influences the ATP and ADP concentrations. In this context, it can probably be concluded that the conversion of ATP by the ATPase reaction was not changed in psoriasis as compared with controls (Table III).

The method used here in the assays of GAPDH gave lower enzymatic levels than those reported earlier (8). An explanation for this difference was not sought, since the assay gave reproducible results.

The GAPDH activity was increased in all four diseases studied; with regard to psoriasis and neurodermatitis this was known earlier (5). The lichen planus lesion displayed activities almost as

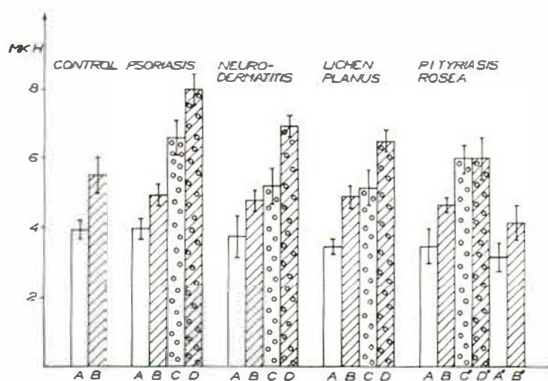


Fig. 1. Means of the activity of adenylate kinase (moles/kg dry weight/hour, MKH). Range bars on each column indicate S.E.M. The letters at the bottom indicate subcorneal (A, A*) and basal (B, B*) epidermis taken from non-involved skin and subcorneal (C, C*) and basal (D, D*) epidermis taken from lesions. Asterisks refer to a biopsy site on the trunk; otherwise biopsies were collected from the forearm.

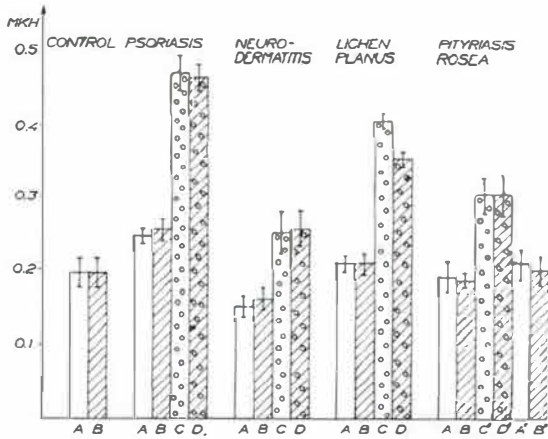


Fig. 2. Means of the activity of glyceraldehydephosphate dehydrogenase. The notation is the same as in Fig. 1.

high as those found in psoriasis (see Fig. 2). As recently shown, the same was evident for G6PDH (6). Both enzymes are located in the cytosol and differ from the intramitochondrial enzyme β -hydroxyacyl-CoA dehydrogenase. The activity of this enzyme was decreased in epidermal layers of the lichen planus lesion (6). Thus, mitochondrial and cytosol enzymes are affected differently in this disease. It may be pointed out that the epidermal glycogen content in the lichen planus lesion is low or has disappeared and that its respiration at least is not increased (14). In spite of the pronounced cellular alterations in basal epidermis, high activities were displayed by the glycolytic and the pentose shunt enzymes. Further studies must be undertaken in order to obtain a more precise scheme of the metabolic consequences in this disorder.

One aim of this study was to find out whether a change of the relative epidermal height was related to enhanced enzymatic activities. No evidence of such a relationship was found, however. The various keratinizing patterns in the diseases investigated could not clearly be associated with variation in enzymatic activities. The high levels of enzymatic activity found in the psoriatic lesion are in agreement with previous findings of increased activities of many enzymes belonging to different metabolic pathways (4). Such alterations are well explained by the increased cell turnover in psoriasis; how these results might be linked to the aetiology of the disease is, however, poorly understood.

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