TABLE 1. Amounts of ATP in HeLa Cells in the Exponential Growth Phase 20 min after their Irradiation with He–Ne Laser in Various Doses (mean±SEM)

| Dose, J/m² | Amount of ATP, % of control level |
|---|---|
| – | 100±2.0 |
| 10 | 100.5±3.5 |
| $10^2$ | 170.8±2.3 |
| $10^3$ | 238.3±5.5 |

Irradiating the cells with the He-Ne laser on day 1 or 2 after seeding was found not to have increased the amount of ATP as compared to intact cells (Fig. 3), whereas irradiating them on day 3, 5, or 8 increased its level; their sensitivity to the radiation remained virtually the same and peaked on day 5 (190%) under the experimental conditions used.

The results of this study indicate that He-Ne laser radiation-induced ATP extrasynthesis does not occur just only in isolated rat liver mitochondria [2,3,7,10]. After He-Ne laser irradiation, ATP also increases in cells cultured *in vitro*.

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# Epidermal Ornithine Decarboxylase Activity in Psoriasis: a Biochemical Indicator of a Hyperproliferative Process

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There has been an upsurge of interest in the synthesis and degradation of polyamines in different biological objects in the last decade in connection with the vital role played by polyamines in the regulation of cell metabolism and in DNA, RNA, and protein synthesis, particularly in the course of malignant growth [5, 7, 11, 14, 15]. Convincing evidence has been obtained of a direct relationship between polyamine concentration of eukaryotic cell differentiation rate. A specific level of polyamines regulated by the ratio of enzymes catalyzing polyamine synthesis and degradation has been found to be characteristic of different stages of cell differentiation and growth [13, 16]. An increased activity of enzymes catalysing polyamine synthesis and, conversely, a decreased activity of enzymes of polyamine catabolism are characteristic biochemical features of some rapidly growing tissues (embryonic and malignant, regenerating liver, etc.) [4, 12]. We should like to mention here our previous

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data which demonstrated the accumulation of polyamines in epidermal tissue and their increased urinary excretion in psoriasis sufferers [1,2,8].

Psoriasis is a highly prevalent skin disease clinically characterized by erythematous plaques and scaling and histologically by specific epidermal hyperproliferation. The etiology and pathogenesis of psoriasis are still obscure. A number of antimetabolites, e. g., retinoids, methotrexate, etc., are widely used in its treatment, with variable success. In our previous papers [8,9] we proposed quite a new approach to the treatment of this disease based on the use of an antagonist of pyridoxal phosphate (ornithine decarboxylase coenzyme, specifically isonicotinic acid hydrazide.

Bearing all this in mind, as well as the direct and significant contribution of polyamines to cell growth and differentiation and their accumulation in the skin in psoriasis, we have attempted to investigate the activity of ornithine decarboxylase, the key eznyme of polyamine biosynthesis, in the epidermis of psoriasis patients.

## MATERIALS AND METHODS

Ornithine decarboxylase (ODC) activity was measured in skin samples taken from involved and intact epidermis of six hospitalized but not yet treated patients. For comparison the enzyme activity was measured in regenerating rat liver (as a hyperproliferative process) and in normal rat liver tissue. For control epidermal samples of normal subjects were examined. Human epidermis samples were taken from sites never exposed to solar radiation or therapeutic UV or IR irradiation. Epidermal flaps were removed surgically with a special keratome, with a shim setting of about 0.125 mm in size [15,16]. The time of epidermal strip removal and enzyme activity measurement had to be minimized because of the very short half-tiome of ODC. That is why the tissue samples were immediately homogenized on ice or in some cases frozen in liquid nitrogen.

The enzyme activity was measured in supernatants of the tissue homogenates in the presence of L-ornithine as a substrate according to the method developed in our laboratory [4]. Protein was assayed after Lowry [10], DNA after Spirin [3].

## RESULTS

Table 1 shows that the mean ODC levels in patients' involved epidermis (1.38±0.41 nmol/h/mg protein) were three times as high as the enzyme levels in normal epidermis (0.43±0.11 nmol/h/mg protein). This difference is still higher if ODC activity is calculated per μg of DNA, surpassing the normal enzyme activity almost sixfold (0.039±0.006 vs. 0.006±0.001 nmol/h/μg of DNA in control intact epidermis). These data indicate, first, that ODC may indeed be a biochemical marker of a hyperproliferative process in the epidermis in psoriasis or of a malignant skin involvement in humans [6], and, second, that measuring the true activity of the enzyme, just as with any other chemical parameter in the skin involved in the psoriatic process, one should express the resultant values on the basis of DNA, because such expression correlates with the counts of live, not dead cells.

The table also demonstrates that the so-called uninvolved of intact skin of psoriasis patients is not at all intact in terms of this biochemical parameter, contrary to the belief of some authorities [11, 12] who did not find any noticeable difference in ODC activity estimated per mg of protein in involved and uninvolved epidermal sites of psoriasis patients. Our data indicate that the enzyme activity on uninvolved skin sites of patients surpasses almost four times the normal values estimated per μg of DNA. On the other hand, we have not revealed any noticeable difference in ODC activity on involved and uninvolved sites of the epidermis of the same psoriasis patients.

We thought it interesting to compare ODC activities in other hyperproliferative tissues. We measured the enzyme levels in normal liver of intact rats and in regenerating rat liver after partial hepatectomy (Table 1). Evidently, for liver tissue there is no need to express ODC activity on the basis of DNA, because the enzyme levels ezpressed both through protein and DNA were almost the same, the only difference being that in regenerating liver tissue (that is,

TABLE 1. Ornithine Decarboxylase Activity in Human Skin in Health and in Psoriasis (Control: Intact and Regenerating Liver Tissue of Rats) ($M \pm m$)

| Tested tissue | ODC level in nmol/h | |
|---|---|---|
| | per mg protein | per μg DNA |
| Normal subjects' epidermis | 0.43±0.11 | 0.006±0.001 |
| Epidermis from uninvolved skin sites of psoriasis patients | 1.34±0.48 | 0.025±0.007 |
| Epidermis from involved skin sites of psoriasis patients | 1.38±0.41 | 0.039±0.006 |
| Normal rat liver | 0.95±0.15 | 0.014±0.003 |
| Regenerating rat liver | 8.52±1.18 | 0.121±0.029 |

in the cells characterized by a high mitotic activity and a high rate of DNA, RNA and protein synthesis) these values were one order of magnitude higher than in intact liver (Table 1).

The epidermal proliferation rate in psoriasis is known to be in strict correlation with the degree of epidermal injury and to approximately correspond to the rate of cell loss. A hyperproliferative process implies the presence of a high concentration of polyamines which may be provided by enzymes catalyzing their rapid synthesis. The present research has demonstrated that the activity of one of the key enzymes in polyamine synthesis, ornithine decarboxylase, was drastically increased during both hyperproliferative processes: in psoriasis and in rat regenerating liver.

Hence, our results permit the inclusion of ODC measurements in the epidermis as an additional enzymatic test for any hyperproliferative process in the skin, including psoriasis. This specific biochemical marker may be useful not only in diagnostic investigations, but in the search for methods of active intervention in the pathological process and of treatment of psoriasis using antimetabolites of ornithine and S-adenosylmethionine decarboxylation products and substrates, as well as competitive and noncompetitive inhibitors of coenzymes (pyridoxal phosphate and pyrrole quinolinoquinone, respectively).

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