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ADENOSINE TRIPHOSPHATASE ACTIVITY AS EVIDENCE FOR PERSISTENCE OF LANGERHANS CELL IN PSORIATIC SCALES

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Abstract. Scales from 50 patients with psoriasis uniformly revealed adenosine triphosphatase activity localized in a random, interrupted, linear manner between the corneocytes. No staining was seen in tape strippings from normal stratum corneum, nor in the scales from a variety of dermatoses. It was seen, however, to a lesser degree in certain inflammatory processes such as atopic dermatitis. In view of the fact that adenosine triphosphatase selectively stains the Langerhans cell in the epidermis, we have tentatively considered this enzyme patterning of psoriatic scale as evidence of the presence of Langerhans cells.

One of the most easily available diagnostic specimens in dermatologic practice is the scale. Yet, to date, its usefulness seems to have been largely limited to the demonstration of superficial fungi and related microflora. The present study arose in an effort to extend the diagnostic reach of those who would "autopsy" a scale and therein read the history of its past.

Using an adenosine triphosphatase stain, we have found a random dendritic patterning of ATPase activity in psoriatic scales. Such activity is not generally present in normal stratum corneum nor in scales from many non-psoriatic inflammatory dermatoses. We believe that this enzyme stain reveals Langerhans cells being shed into the psoriatic stratum corneum. Additional study will be necessary to confirm the specificity and diagnostic value of this finding.

METHODS

Intercellular (membrane) adenosine triphosphatase activity was localized by the technique of Wachstein & Meisel (10) and variations thereof. In the case of scales, these were collected either by adhesive tape stripping (Scotch tape no. 136, double stick, adherent on a glass microscope slide), or by dull scalpel scraping of scales onto double stick tape slide (4). The entire slide was incubated in an adenosine triphosphate solution for 10–50 min, at 37°C. It was then rinsed in distilled water for 5 min and developed in 0.5% ammonium sulfide solution for 10–20 min. After transfer to distilled water for 15–30 min, the tape was permanently mounted in glycerine jelly. ATPase activity is reflected by a black deposit of lead sulfide.

Control studies on the distribution of adenosine triphosphatase were done on epidermal sheets separated mechanically after an overnight soaking of human or guinea pig skin biopsies in 2 N sodium bromide solution. Comparable studies were made on shave epidermal biopsies (1) and on frozen sections. In these instances the specimens were incubated for 90 min in adenosine triphosphate solution at 37°C. Subsequent rinsing for 5 min in distilled water was followed by 5 min exposure to 0.5% ammonium sulfide. Final rinsing in distilled water was carried out for 15–30 min. Mounting again was done in glycerine jelly.

The substrate medium of adenosine triphosphate had the following composition:

10 ml	125 mg/100 ml adenosine 5 triphosphate disodium salt (Sigma)
10 ml	Tris buffer pH 7.2
	Tris maleate 5 ml 1 m (121 g/l)
	Maleic acid 5 ml 1 M (116 g/l)
	Sodium hydroxide 10 ml 0.5 N (20 g/l)
2.5 ml	0.1 M Mg So$_4$ (12 g/l)
1.5 ml	2% Lead nitrate
1.0 ml	Distilled water. Filter before use.

RESULTS

Fig. 2 shows the patterning of ATPase staining in untreated psoriatic scale. The stain localizes between corneocytes as a striking black linear deposit of lead sulfide. At times, beading is noted and in some places the staining band widens to become more prominent (Fig. 3). The staining is

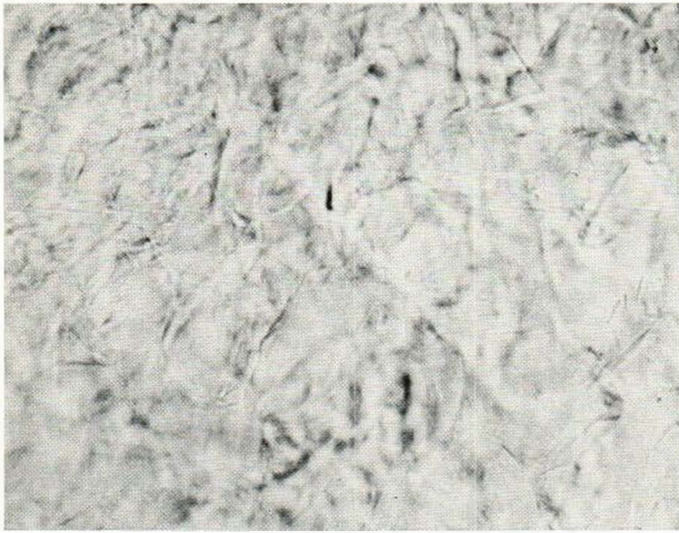

Fig. 1. Absence of staining in scales from inflammatory dermatosis. Adenosine triphosphatase stain, × 440.

not uniform but the strands of ATPase activity are invariably between the keratinocytes and randomly distributed. In certain areas the activity may be so rich as to appear to form a network outlining many of the keratinocytes. The stain remained stable for months.

This ATPase activity as found in the scales in every one of 50 successive patients with psoriasis. Thirty to 40 serial strippings from a single plaque of psoriasis all showed the phenomena on each slide. Although staining is not present in every scale, it was observed in the scales of each of 12 topographic areas studied. It was also demonstrated in 2 cases of pustular psoriasis. This ATPase activity could not be demonstrated in scotch tape stripped stratum corneum of any of 10 normal volunteers. Nor was it present in the stratum corneum exfoliating after a chemical burn,

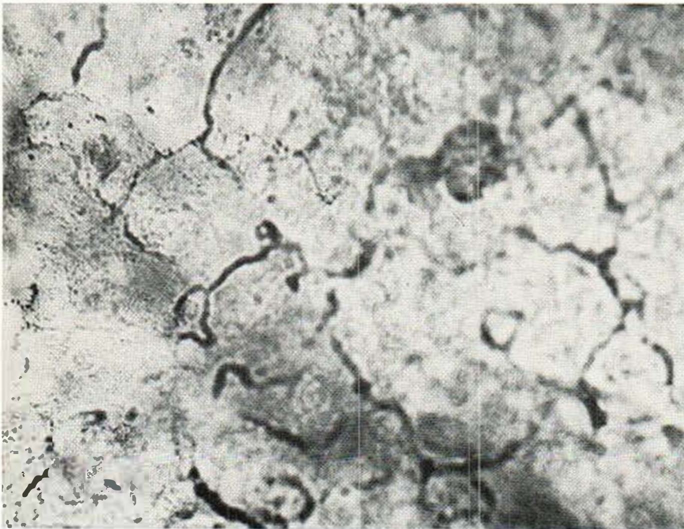

Fig. 2. Intercellular dendritic staining regularly seen in psoriatic scale. Adenosine triphosphatase stain, × 440.

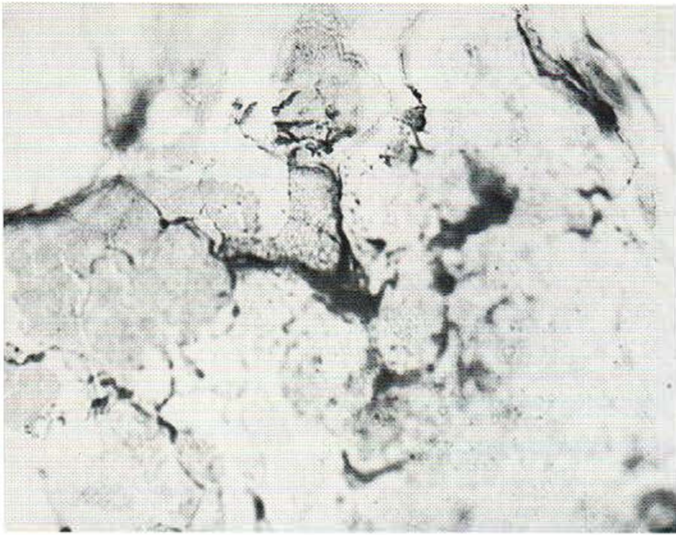

Fig. 3. Cellular patterning of stain in psoriatic scale. Note similarity to Langerhans cell stain (Fig. 4). Adenosine triphosphatase stain, × 440.

sun burn, ultraviolet light or a drug reaction. It could not be found in the scales of tinea pedis (5 cases), (Fig. 1), although the fungi showed a dramatically high ATPase activity (Fig. 5). Five cases of ichthyosis and 5 of seborrheic dermatitis failed to show ATPase activity in the scale. Isolated observations on the scales of actinic keratosis, tinea versicolor, pityriasis rosea, lichen sclerosus et atrophicus, also failed to reveal this ATPase patterning.

However, in 10 cases of atopic dermatitis, 6 showed occasional strands of ATPase activity, but never to the degree observed in psoriatic scale. The remaining 4 showed no activity. Positive stains were also obtained in miscellaneous patients with guttate parapsoriasis, pityriasis rubra pilaris, ex-

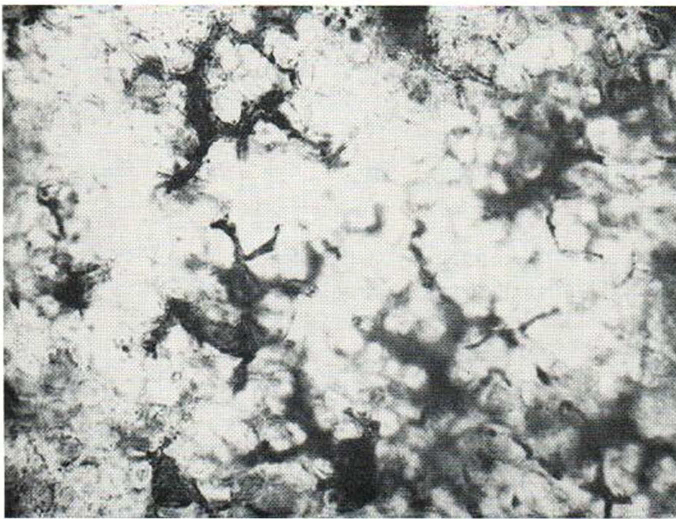

Fig. 4. Langerhans cell staining in normal epidermis. Adenosine triphosphatase stain, × 440.

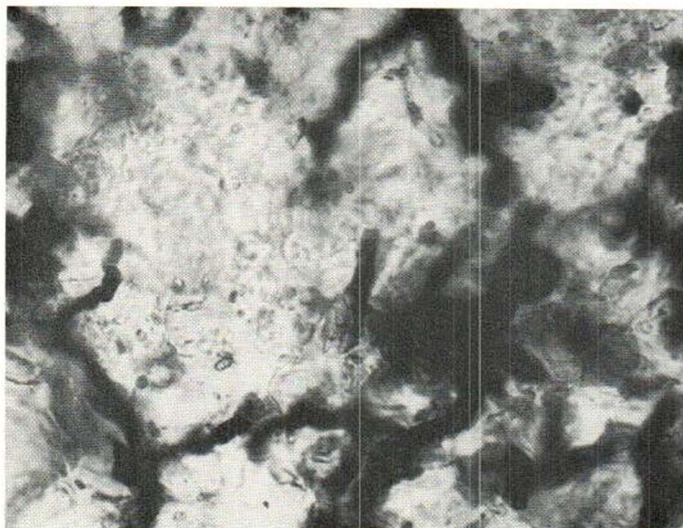

Fig. 5. Fungal hyphae in scale from tinea pedis. Adenosine triphosphatase stain, ×440.

foliative dermatitis, and erythema annulare centrifugum.

In the psoriatic patients, extremely thick scales were not suitable for staining. The ATPase activity was observed in scales from sites which had been treated with topical steroids or tars, as well as from untreated sites. However, it was our feeling that anthralin treatment may block the staining. No matter what the treatment, local or systemic, as the psoriatic lesions involuted the ATPase stain became less evident and finally completely disappeared. Islands of linear ATPase activity could regularly be seen on viewing large sheets of scale from certain psoriatics. These sites appeared to correlate with micropapular foci of psoriatic efflorescence. Stratum corneum from the intervening zone was negative and this was consonant with the feeling that not all scales scraped or stripped from psoriatic lesions showed this distinctive ATPase stain. Shave epidermal biopsies from psoriatics failed to reveal a unique Langerhans cell patterning or staining, although the blood vessels, which stain well for ATPase, were remarkably prominent.

The ATPase staining pattern in the scales could not be duplicated by osmium zinc oxide staining, methylene blue staining, the dopa stain, or an alkaline phosphatase stain. Silver impregnation technique likewise failed to show this pattern.

Varying salts of adenosine triphosphate were employed as the substrate. The best was found to be sodium, but good results were also obtained with the disodium and the dipotassium salt. The barium, calcium and magnesium salts were not satisfactory. It was possible to use the di-ethanolamine as well as the di Tris (hydroxyl methyl) amino-methane salt.

Fig. 4 shows the control studies done to demonstrate the ATPase stained Langerhans cell found in normal epidermis. These studies were done on 20 human volunteers and 20 guinea pigs. In every one, the prominent cell bodies and interlacing dendrites were well seen with this stain. In the more superficial levels of the epidermis the dendritic elements are more prominent than the cell bodies.

DISCUSSION

Interpretation of the significance of the strange fragmented linear patterning seen in Figs. 2 and 3 is difficult. One might immediately assume that it is artefactual. In this ATPase stain the scale is soaked in adenosine triphosphate in the presence of lead so that phosphate hydrolysed by any phosphohydrolase present combines with the lead. After rinsing out of the reagents, any residual lead phosphate is converted to insoluble lead sulfide by immersion in dilute ammonium sulfide. This permits the recognition of the ATPase sites. It is

apparent that any non-enzymatic hydrolysis of the ATP ill be reflected in erroneous attributions. Indeed, the complexity of this area has been recently critically reviewed by Tice (9). Nonetheless, the Wachstein-Meisel stain used by us remains a standard technique (7) for demonstrating ATPase. Our selective demonstration of the Langerhans cell in 40 control epidermal shave biopsies (Fig. 4) added to our confidence in the method as employed by us. Controls were also done using staining media with varying ingredients absent. These were entirely negative. Likewise other stains such as neotetrazolium, dopa, alkaline phosphatase, as well as methylene blue failed to show a non-specific type deposit in these sites. Interestingly, mercury is a known inhibitor of ATPase. Hence it is possible that patients treated with local mercurial preparations might fail to show the distinctive staining we have seen. It would be interesting to study the effect of vitamin D_2, beryllium, detergents and other known inhibitors of ATPase. Certainly it was our finding that anthralin interfered with the stain and this may have been the result of enzyme inhibition.

Inasmuch as the staining occurred in a linear pattern which appeared to run between the scale keratinocytes (corneocytes), one has to consider that it may be the keratinocyte membrane, the intercellular space, or the intercellular cement substances which are stained (15). The fragmented nature of the staining leads us away from the view that the keratinocyte wall is stained. Far more uniform retiform staining would be anticipated if the keratinocyte were responsible for the staining. The possibility of deposits occurring in the intercellular space is rendered plausible by the observation of Mercer & Maibach who have demonstrated that this space is enlarged in psoriatic epidermis, although not in psoriatic scale (5). In this regard, Sugar et al. (8) demonstrated an increase in intercellular membrane-bound ATPase activity with deposits of lead in the distended intercellular spaces which occur in experimentally induced skin cancer in the mouse. Again, however, the discontinuity of staining in our study speaks against this, as well as against the possible staining of an intercellular cement substance. Nonetheless, it is important to point out that the membrane phospholipid, phosphatidylserine specifically activates ATPase (11). Possibly in psoriasis such a phospholipid is present in large amounts. To date we have no evidence of this and experiments with this compound failed to accentuate or alter the staining we produced.

The most attractive interpretation to us is based on Wolff's demonstration (12) that the Langerhans cell specifically stains for ATPase. Although the specificity was not initially observed by an originator of the stain (2) and has been disavowed more recently (16, 17), Wolff's group still presents good evidence that ATPase can be used as a reasonably reliable marker for the Langerhans cell, at least under certain conditions and in the absence of electron microscopy (3, 13, 14). We feel the similarity of staining in the scale and that of the classical Langerhans cell in the epidermis (Fig. 4) suggests that in any rapidly proliferating inflammatory epidermal tissue the sequential desquamation may carry away Langerhans cells, shriveled in shape, but still carrying an active complement of ATPase. It is this enzyme we view as a marker.

Psoriasis is the classic example of rapid epidermal turnover and, with enzyme activities measuring as much as 10 times normal, it is not surprising to us that the psoriatic scale reveals Langerhans cells as dendritic elements persisting between the keratinocytes. Other scaling diseases present only a diminutive form of this remarkable epidermopoiesis and in turn a lesser degree of ATPase activity in their scales. Hence, although the change is not limited to psoriasis, the degree and extent of ATPase staining of scales from untreated lesions may be a helpful diagnostic feature in identifying the presence or absence of psoriasis.

The staining is not as regular and easily photographed as that seen in sections. One must recall that the scale viewed from above does not present as an ideal flat plane. Nevertheless, the stain is easily read under medium power.

Previous studies of ATPase activity have been limited to normal skin and a few diseases. In his review, Niebauer (6) has pointed out that the dendrites of the Langerhans cell extend up to the stratum granulosum but not beyond it. We would agree that for normal tissue this is true, but in psoriasis it is our view that the cells and dendrites are swept out into the stratum corneum.

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