A study of cell proliferation kinetics in the small intestinal epithelium of psoriasis patients

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Summary
Small intestinal biopsies from psoriasis patients and from normal controls were obtained with a multipurpose suction tube. The tissue was incubated with tritiated thymidine and processed for autoradiography.

Labelling indices (LI) were calculated by counting labelled cells in all crypt cross-sections through the entire proliferative compartment. LI was increased in psoriatics as compared to controls indicating alterations in small intestinal epithelial cell kinetics in psoriasis.

A continuous labelling allowing us to calculate the S-phase duration and the total cell turnover time was probably not obtained within an experimental period.

Introduction
It has been disputed whether the non-specific enteropathy frequently found in psoriatics is linked to that particular disease or belongs in the 'dermatogenic enteropathy' group (Barry et al., 1971; Roberts & Preston, 1971; Shuster, 1968; Marks & Shuster, 1970).

In a recent work we claim to render probability of the existence of a malabsorption in psoriasis (Hendel et al., 1982).

The aim of the present work is to explore the aetiology of an intestinal malfunction similar to that found in psoriasis.

Material and methods
Five psoriasis patients and five control persons with no gastrointestinal disorder were studied.

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The psoriasis group consisted of two women and three men, aged 21–64 years. None of the patients suffered from any other disease than psoriasis or had ever experienced any kind of systemic psoriasis treatment.

The normal controls were four women and one man, aged 35–60 years.

Biopsies from two patients with dermatitis herpetiformis and one patient with gluten-sensitive enteropathy served as method controls.

The biopsy procedure took place in the morning after an overnight fast. A multipurpose hydraulic suction biopsy tube was used with the tip placed immediately beyond the ligament of Treitz. The position was checked by fluoroscopy.

At least ten biopsies were removed from each patient. The biopsies contained mucosa to the level of the mucosal muscular layer. The biopsies from psoriasis patients revealed surface architecture ranging from group I to group IV (Lee & Toner, 1978), while the normal controls all belonged to group I.

**Autoradiography**

The biopsy specimens meant for cell kinetic evaluation were shortly rinsed in unlabelled medium and cut into pieces of approximately 1 mm³. Immediately thereafter the specimens were incubated in a shaking waterbath at 37°C in 5 ml of tissue culture medium (Eagles Minimal Essential Medium (MEM, GIBCO) with Earle’s salt, fetal calf serum 10%, penicillin-streptomycin solution 100 units/ml and L-glutamine 4 mM) containing 20 μCi of tritiated thymidine (Radiochemical Centre, Amersham, England, specific activity 23–26 Ci/mM). pH was adjusted to 7.4 with Hepes buffer.

In all thirteen individuals, two specimens from each were incubated for 30 min, and two for 60 min. From each of four psoriasis patients and four controls, two specimens were incubated for 120 min. From each of three psoriasis patients and two controls, two specimens were incubated for 180 min. One specimen from each individual was incubated for 60 min, in unlabelled tissue culture medium. Specimens from two patients with dermatitis herpetiformis were incubated for 120 min as well.

After incubation, the tissue was washed in unlabelled culture medium for 5 min, fixed in Bouin’s solution for 1 h, embedded in paraffin and serially sectioned at 5 μm. The slides were dipped in photographic emulsion (Ilford K2) and exposed for 2 weeks. After development, the slides were stained with haematoxylin.

**Evaluation technique**

The epithelial cells were considered labelled if they had five or more grains over the nucleus. The number of labelled cells and the total number of cells in all suitable cross-sections of the crypts containing at least one labelled cell were counted (Hart Hansen, Pedersen & Larsen, 1975) (Fig. 2). A cross-section was considered suitable if the length was not more than twice the width and the epithelium was monolayered.

The labelling index (LI) is defined as the proportion of labelled cells in a given cell population.

Every fifth section was surveyed and at least 2000 epithelial cells in each sample were counted, and a mean LI was calculated.
Cell counts were performed blindly by two investigators. The LI was calculated as an average of determinations in two tissue samples, each counted by two observers.

When counting only cross-sections containing labelled cells we are sure to be within the proliferative compartment of the crypts. On the other hand, by not counting cells in cross-sections without labelled cells we might not include all epithelial cells in the proliferative compartment and hence overestimate the LI. To reduce this error, a correction equation has been worked out (Hart Hansen et al., 1975). However, considering the average number of epithelial cells in the crypt cross-sections together with the calculated LI we find the need of a correction to be negligible.

For statistical analysis the Mann-Whitney U-test was used.

Results

If the LI was markedly declining with increasing incubation time, the biopsy in question was discarded. Two specimens from one psoriasis patient (incubation period 2 h) were thus excluded.

The number of biopsies (patients) qualified for interpretation appears from Fig. 1.

**Figure 1.** The percentage of labelled cells (labelling index, LI) v. incubation time. The psoriasis group and the normal controls are easily distinguished. The rate of incorporation of tritiated thymidine from 0.5 to 1 h is not significantly different ($P > 0.05$, Mann-Whitney U-test) in the two groups, only the proportion of labelled cells in the psoriasis group is on a higher level. (●) psoriasis; (○) control.
The 'background' labelling in our laboratory did hardly exceed 2 grains over the nucleus in unlabelled tissue sections processed for autoradiography. The 'background' in the labelled preparations, counted as grains over the villus epithelium, did not exceed one grain per 200 \( \mu \text{m}^2 \).

The labelling indices are calculated as an average of determinations in two tissue samples, each counted by two observers (Table 1).

Dermatitis herpetiformis and gluten-sensitive enteropathy are diseases characterized by

<table>
<thead>
<tr>
<th>Labelling indices (LI) (%)</th>
<th>0.5*</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>13</td>
<td>17</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>20</td>
<td>26</td>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td>Dermatitis herpetiformis</td>
<td>22</td>
<td>28</td>
<td>29</td>
<td>—</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>28</td>
<td>35</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Incubation time (h).
increased epithelial cell turnover in the small intestines and may, as such, serve as method control (Wright et al., 1973, 1979).

In the psoriatic small intestine a significantly higher LI was found as compared to normal controls ($P < 0.01$, Mann-Whitney U-test).

The grain counts (grains/nucleus) is similar in psoriasis and normal controls, while a much heavier labelling is found in dermatitis herpetiformis and gluten-sensitive enteropathy.

Labelled mitoses were not observed.

**Discussion**

In psoriasis, an enhanced cell proliferation in the germinative layers of the skin leading to epithelial dysfunction has been suggested (Goodwin, Hamilton & Fry, 1973; Ralfs et al., 1981).

As shown in a previous study, psoriasis patients are often subject to a slight non-specific enteropathy different from the so-called 'dermatogenic enteropathy' (Hendel et al., 1981). We suggested this enteropathy to be an epithelial dysfunction due to cellular immaturity.

It is known that increasing the reproduction rate of a certain cell population might influence various cell qualities (Rijke et al., 1976; Gudmand-Hoyer et al., 1978). Using clinical routine test, we were not able to reveal a distinct pattern in the psoriatic enteropathy, but these tests might not be sensitive enough when dealing with a very delicate problem or might misfit affected qualities.

To look directly into the problem of a changed rate of proliferation, we used the method of Hart Hansen et al. (1975). The method was created to study human gastric mucosal proliferation in vitro, but has turned out to be useful in the small intestine as well (Hart Hansen, Larsen & Svendsen, 1978).

A certain overestimation of the LI might occur by not considering any cross-section with no labelled cells. But using the nomogram for correction, we found the error negligible.

When only cross-sections containing at least one labelled cell is taken into consideration, we are sure to work entirely within the proliferative compartment and the growth fraction is thus 100%.

The method is, of course only dealing with changes within the proliferation zone. We are not able to draw any conclusions whether changes in the proportion of the proliferative compartment do occur. Still we are confronted with a significantly higher LI in psoriasis than in normal controls. This might be due to a prolonged S-phase and/or a decreased cell cycle time leading to an altered total cell turnover. On the assumption that we are dealing with 'steady state' cell kinetics and the DNA labelling rate is constant, the S-phase duration ($T_s$) can be calculated from the equation $T_s = LI/r_s$, where $r_s$ is the rate of cell entry into S-phase and LI the initial labelling index. From our results it is difficult or impossible to determine whether a continuous labelling ever arises or the 'pulse' labelling is continuing during the full experimental period.

Recently, Fluge & Aksnes (1981) have experienced similar difficulties and have described in detail a rather dynamic proliferative behaviour in cultured duodenum.

If the increase of LI with incubation time under our experimental conditions is uncritically taken as an expression of $r_s$, we find a $T_s$ much too short to be correct. This applies to all incubation intervals in samples from two psoriasis patients, while the calculations might be valid
in normal controls considering the incubation period from 2 h to 3 h. If we use the intinitally measured LI (0.5 h incubation), we get a $T_s$ of about 5 h.

If the 'initial LI' is defined as the $y$-intercept of the (extrapolation) line drawn through the LI$_{in}$ and the LI$_{ih}$ values we get $T_s$ of approximately 9 h.

Thus, we can hardly claim to have achieved an overall stable DNA-labelling under in vitro experimental conditions.

This observation is supported by some preliminary results from our laboratory concerning a comparison of in vivo and in vitro labelling of mouse small intestine. It seems that the 'in vitro LI' first will reach the 'in vivo LI' after approximately 2 h of incubation.

The similarity between grain counts per nucleus in psoriasis and controls and the much higher grain counts in dermatitis herpetiformis and celiac disease is a problem we have not explored further. However, this finding connected with the higher LI in psoriasis might support the theory of a decreased cell cycle time in the psoriatic small intestinal epithelium.

**Conclusion**

In our experiments we find psoriasis patients to have an increased LI in their small intestine when compared to normal controls.

This indicates cell kinetic alterations in the small intestinal epithelium of psoriatics.

The recognition of changes in cell kinetics in organs different from the skin in psoriasis may stimulate the interest in systemic treatment of this disease.

**Acknowledgment**

We thank Jette Christiansen for her skilful assistance concerning the histologic preparation of the tissue and Eva Hoffmann for her tedious work as one of the two observers in the counting procedure. Inger Johansson, who has saved us the bother with laborious typing, deserves to be thanked as well.

**References**


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