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# Cytokinetics of Epidermic Cells in Skin from Human Cadavers

## II. Dependency on Sex, Age, and Site

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**Summary.** Kinetic data on the labeling index (LI), DNA synthesis time ($t_s$), and potential doubling time ($t_{pot}$) of epidermic cells in relation to sex, age, and site were obtained by in vitro incubation of skin cylinders from 45 human cadavers with DNA precursors $^3$H- and $^{14}$C-thymidine. In a first study on parts of the same material, it was established that LI over a period of more than 70 h and $t_{pot}$ over a period of at least 30 h remained essentially unchanged and are comparable with live humans, when the cadavers were stored at 4°C. The following results were obtained: The female and male cadavers had a LI of 2.6% (± 0.8%) or 2.5% (± 0.8%), a $t_s$ of 3.9 h (± 0.2 h) or 5.0 h (± 1.6 h), and a $t_{pot}$ of 168.5 h (± 34.3 h) or 183.9 h (± 27.2 h). The LI for the thigh and knee ranged between 21.3% and 25.8% in different age groups. No statistically relevant differences were established between the sexes or among the age groups. Topographic allocation of the proliferative-kinetic data ultimately showed that, on the average, LI was relatively high at the elbow (3.1% ± 1.0%) with short $t_{pot}$ (109.3 ± 72.5 h) and a comparatively large epidermal diameter (47.1 μm); by contrast, LI at the lower abdomen was impressively low (2.1% ± 0.8%), $t_{pot}$ relatively long (183.0 ± 138.7 h) and mean epidermal diameter relatively small (23.0 μm). Nevertheless, no statistically relevant differences were established between data for elbow and lower abdomen or between other data for different sites. The proliferative-kinetic data for human cadavers were compared with data reported in the literature for live humans.

**Key words:** Epidermis, cell kinetics – DNA synthesis time – Kinetic data of epidermic cells

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**Zusammenfassung.** Durch In-vitro-Inkubation von Hautstanzen menschlicher Leichen mit den DNS-Vorläufern $^{3}H$- und $^{14}C$-Thymidin sollten kinetische Daten zum Markierungsindex (MI), zur DNS-Synthese-Zeit ($t_s$) und zur potentiellen Verdoppelungszeit ($t_{pot}$) epidermaler Zellen in Abhängigkeit von Geschlecht, Alter und Topographie gewonnen werden. Folgende Ergebnisse konnten erhalten werden: Frauen und Männer hatten jeweils einen MI von 2.6% (± 0,8%) bzw. 2,5% (± 0,8%), – eine $t_s$ von 3,9 h (± 0,2 h) bzw. von 5,0 h (± 1,6 h) – und eine $t_{pot}$ von 168,5 h (± 34,3 h) bzw. von 183,9 h (± 27,2 h). Für unterschiedliche Altersklassen lagen die MI zwischen 21,3 und 25,8%, bei Biopsieentnahme aus Oberschenkel und Knie. Statistisch relevante Unterschiede bestanden weder zwischen Geschlechtern noch zwischen den Altersklassen. Bei topographischer Zuordnung der proliferationskinetischen Daten konnte schließlich festgestellt werden, daß der MI im Ellenbogen mit 3,1% (± 1,0%) im Mittel relativ groß war bei gleichzeitig bestehender kurzer $t_{pot}$ von 109,3 h (± 72,5 h) und vergleichsweise großer Epidermisdicke von 47,1 µm. Demgegenüber war im Unterbauch der MI mit 2,1% (± 0,8%) auffällig niedrig, die $t_s$ mit 2,9 h (± 1,3 h) relativ kurz und die $t_{pot}$ mit 183,0 h (± 138,7 h) relativ lang, – bei einer mittleren Epidermisdicke von 23,0 µm. Ein statistisch relevanter Unterschied bestand weder zwischen den Daten von Ellenbogen und Unterbauch noch zwischen den übrigen Daten verschiedenster Topographie. Die an der Leiche beobachteten proliferationskinetischen Daten werden mit Informationen verglichen, die über lebende Menschen im Schrifttum zu identischen Fragen vorliegen.

**Schlüsselwörter:** Epidermis, t Zellkinetik – DNS-Synthesezeit, Geschlechtsabhängigkeit epidermaler Zellen

## Introduction

The results of cytokinetic investigations of epidermis cells from human cadavers have been reported elsewhere [15]: skin biopsies taken from the extensor side of the thigh were investigated by in vitro incubation with radioactively labeled thymidine; the following postmortal interval (PMI)-dependent kinetic data were obtained by counting 1,000 cells from the basal cell layer of each skin specimen:

| | | |
|---|---|---|
| Labeling index (LI): | 2.71 ± 0.76% | (PMI, approx. 20 h) |
| | 2.80 ± 0.91% | (PMI, approx. 40 h) |
| DNA synthesis time ($t_s$): | 4.75 ± 1.44 h | (PMI, approx. 30 h) |
| | 3.20 ± 1.10 h | (PMI, approx. 40 h) |
| Potential doubling time ($t_{pot}$): | 181.30 ± 57.2 h | (PMI, approx. 30 h) |
| | 137.70 ± 40.4 h | (PMI, approx. 40 h) |

The LI and $t_{pot}$ values, especially in the early PMI, compared well with cytokinetic observations in the epidermis of live humans; the $t_s$ for the cadavers, however, tended to be short. No statistically relevant differences between cadavers and live humans, however, could be established.

Since systematic investigations on sex, age, and site dependency of epidermic cell cytokinetics are virtually impossible in live humans, it seemed appropriate, based on the above mentioned finding, to carry out such systematic studies on cadavers. These investigations describe both relative and absolute differences comparable with in vivo conditions.

## Material and Methods

The material and methods are described briefly in the present study (for details see [15]).

Biopsy cylinders (0.3 mm) were taken from the temple, anterior part of the neck, mamma (upper outer quadrant), lateral chest wall, lower abdomen, thigh (extensor side), knee (extensor side), upper arm (flexor side), and elbow of 45 selected cadavers. For various reasons, the biopsies were not always taken in the same way in all cadavers; in some cadavers, specimens were removed twice at different PMIs.

The cadavers were selected according to the following criteria:

1. Definable time of death ± 2.2 h,
2. Absence of intoxication or cytostatic therapy,
3. Absence of skin disease.

A detailed description of a part of the case material is presented in the study cited above [15]. Ten additional cases are integrated in the present study.

The specimens were incubated according to Helpap and Maurer [8], see also [9, 10, 13]: some only once in a solution containing $^{3}$H-thymidine ($^{3}$H-TdR) at a concentration of 20 µCi/ml (specific activity, 5 Ci/mmol; Code No. TRA 61, Amersham Buchler, Braunschweig, FRG). Hundred fourteen specimens were incubated twice; the second solution contained $^{14}$C-thymidine ($^{14}$C-TdR) at a concentration of 1 µCi/ml (specific activity, 54 mCi/mmol; Code No. CFA 532, Amersham Buchler, Braunschweig, FRG). All biopsy specimens were incubated immediately after removal at 37°C under 2.2 atm $O_2$. After incubation, the specimens were fixed in 4% paraformaldehyde with added cold thymidine (1 mg/lm; Code No. 18600; Serva Feinbiochemica, Heidelberg, FRG) to inhibit further incorporation of radioactive thymidine.

The paraffin-embedded specimens were cut into 5 µm-thick sections. Photographic processing was made by the dipping method (film, Kodak NTB2 or Ilford K2) with an exposure time of 14 days. Thousand cells from the germinal layer were counted per specimen; nuclei were considered labeled if they contained at least four reduced silver granules or at least two tracks that originated at the cell nucleus. The background was extraordinarily low and, therefore, could be neglected. No excretory ducts of sweat glands or hair follicles were counted.

The synthesis time was calculated according to Schultze [21]; the potential doubling time, according to Maurer and Schultze [14]. The data were analyzed statistically by Student's *t*-test [20]; a difference at the 1% level was considered significant.

In addition, the epidermal diameter was measured using a micrometer eyepiece; the mean epidermal diameter (excluding the horny layer) was based on five measurements.

## Results

### *Sex Dependency*

LI, $t_s$, and $t_{pot}$ was determined in biopsies of thigh skin, independent of the postmortal storage time (Table 1, Fig. 1). LI was virtually the same in both sexes. No significant differences were established between mean $t_s$ and $t_{pot}$ values.

### *Age Dependency*

Age dependency was assessed by evaluating three age groups (15 years and younger, 20–60, 70 and older). Thigh and knee biopsies were examined sepa-

**Table 1.** Sex and kinetic data of epidermic cells

| Kinetic data | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | *N* | $\bar{x}$ | s | *N* | $\bar{x}$ | s |
| LI (%) | 17 | 2.6 | 0.8 | 28 | 2.5 | 0.8 |
| $t_s$ (h) | 7 | 3.9 | 0.2 | 4 | 5.0 | 1.6 |
| $t_{pot}$ (h) | 7 | 168.5 | 34.3 | 4 | 183.9 | 27.3 |

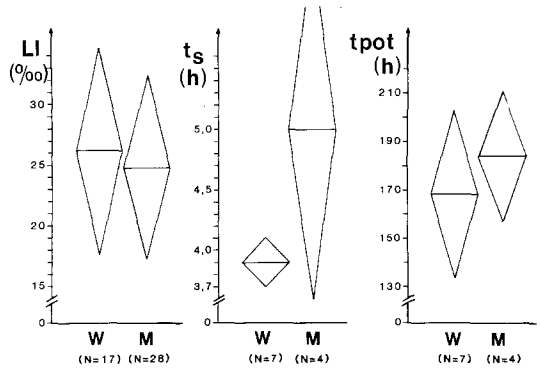


**Fig. 1.** Sex dependency of labeling index, DNA synthesis time, and potential doubling time (median = *horizontal* line; standard deviation = *triangle above* and *below* horizontal line)

**Table 2.** Age dependency of labeling index (‰) in skin form thigh and knee with a postmortal interval of less than 24 h

| Age (yr) | Labeling index (‰) | | | | | |
|---|---|---|---|---|---|---|
| | Thigh | | | Knee | | |
| | *N* | $\bar{x}$ | s | *N* | $\bar{x}$ | s |
| ≥ 15 | 7 | 28.7 | 14.5 | 6 | 25.2 | 8.3 |
| 20–60 | 15 | 21.3 | 10.2 | 14 | 28.5 | 9.9 |
| ≤ 70 | 13 | 25.8 | 9.6 | 10 | 21.3 | 4.9 |

rately; only biopsy material obtained within 24 h after irreversible cardiac arrest was evaluated. The findings are presented in Table 2 and Fig. 2.

No significant differences were established in the individual age groups. No relevant differences were found between the LI of thigh skin and that of knee skin. Since double labeling was made in comparatively few cases, the age dependency of $t_s$ and $t_c$ was not calculated.

*Location*

The LI for skin from various parts of the body was determined independent of PMI (cp. Table 3, Fig. 3). The value for the lower abdomen (LI, 2.1% ± 0.8%) is comparatively low, and the value at the elbow (LI, 3.1% ± 1.0) is relatively high. No significant difference, however, could be established between these or other locations.

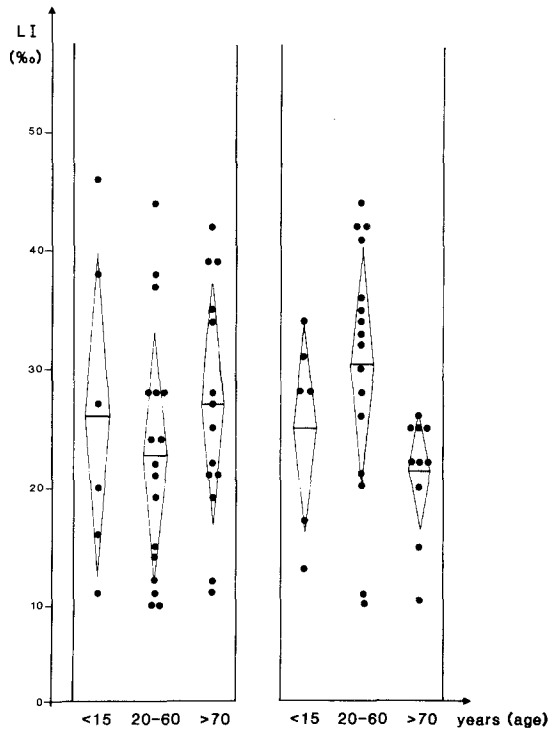


**Fig. 2.** Age dependency of labeling index of epidermic cells from thigh (*left* group of columns) and knees (*right* group of columns; median = *horizontal* line; standard deviation = *triangle above* and *below* horizontal line; single values = *circle*)

The mean $t_s$ values are listed in Table 3 and in Fig. 4 according to the topographic distribution of the biopsy site. The lowest value ($2.94 \pm 1.33$ h) was found in the lower abdomen and the highest ($6.3 \pm 3.6$ h) in the upper arm. No significant difference, however, could be established.

Calculation of $t_{pot}$ (Table 3) revealed an impressively short interval at the elbow ($t_{pot}$, $109.3 \pm 72.5$) and a long interval at the upper arm ($t_{pot}$, $242.8 \pm 123.1$). No statistically significant difference could be established between these extreme values or other biopsy sites.

The mean values obtained for a site-dependent measurement of epidermal diameter are presented in Table 3 and are allocated to the corresponding LI, $t_s$, and $t_{pot}$ values. The lowest LI correlated with the smallest epidermal diameter (lower abdomen) as well as the highest LI with the largest epidermal diameter (elbow).

## Discussion

The problem of comparing observations in cadavers with those in live humans has already been discussed (cp. [15]): The allocation of the data from the same case material to the PMI, which has been reported elsewhere, showed a PMI-dependent shortening of $t_s$ and $t_{pot}$; these changes, however, were not significant. The LI for cadavers stored at 4°C, even for those stored longer 70 h, remained virtually unchanged. The range of mean LI and $t_{pot}$ values corresponded

**Table 3.** *Location and mean labeling index, DNA synthesis time, and mean potential doubling time*

| Location | LI (%) | | | $t_s$ (h) | | | $t_{pot}$ (h) | | | Epidermal diameter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *N* | $\bar{x}$ | s | *N* | $\bar{x}$ | s | *N* | $\bar{x}$ | s | *N* | $\bar{x}$ | s |
| Temple | 23 | 2.7 | 0.8 | 16 | 3.5 | 1.9 | 16 | 127.0 | 64.9 | 23 | 33.7 | |
| Anterior part of neck | 22 | 2.7 | 0.9 | 16 | 4.3 | 2.1 | 16 | 165.0 | 104.3 | 22 | 28.0 | |
| Mamma | 26 | 2.6 | 1.0 | 16 | 4.1 | 2.4 | 16 | 173.6 | 116.1 | 26 | 24.9 | |
| Lower abdomen | 25 | 2.1 | 0.8 | 10 | 2.9 | 1.3 | 10 | 183.0 | 138.7 | 23 | 23.0 | |
| Chest wall | 25 | 2.3 | 1.1 | 14 | 3.7 | 1.6 | 14 | 180.4 | 110.0 | 25 | 25.0 | |
| Thigh | 24 | 2.3 | 1.0 | 15 | 4.2 | 1.5 | 15 | 167.0 | 55.0 | 24 | 30.4 | |
| Knee | 14 | 2.5 | 0.5 | 5 | 3.6 | 1.6 | 5 | 138.1 | 79.4 | 14 | 41.8 | |
| Upper arm | 27 | 2.4 | 1.0 | 16 | 6.3 | 3.6 | 16 | 242.8 | 123.1 | 27 | 27.8 | |
| Elbow | 14 | 3.1 | 1.0 | 6 | 3.4 | 1.8 | 6 | 109.3 | 72.5 | 14 | 47.1 | |

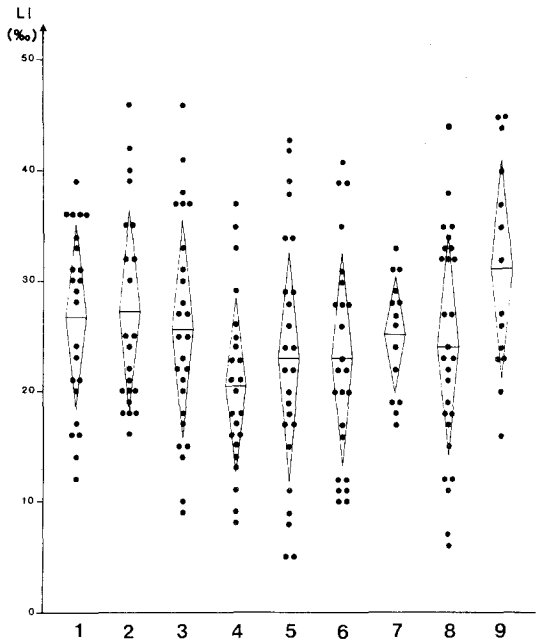


**Fig. 3.** Site dependency of labeling index: Column 1 = temple; column 2 = anterior part of neck; column 3 = mamary gland (*upper outer* quadrant); column 4 = *lower* abdomen; column 5 = *lateral* chest; column 6 = thigh (extensor side); column 7 = knee; column 8 = *upper* arm; column 9 = elbow (median = horizontal line; standard deviation = triangle above and below horizontal line; single values = circle)

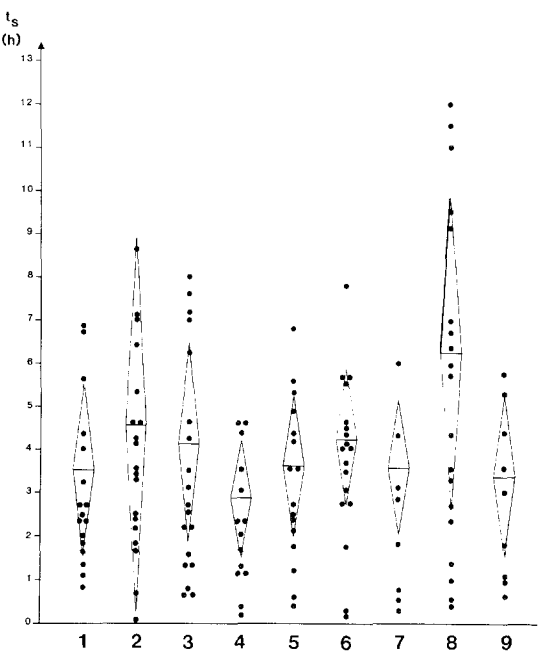


**Fig. 4.** Site dependency of DNA synthesis time: Column 1 = temple; column 2 = anterior part of neck; column 3 = mamary gland (*upper outer* quadrant); column 4 = *lower* abdomen; column 5 = *lateral* chest; column 6 = thigh (extensor side; column 7 = knee; column 8 = *upper* arm; column 9 = elbow (median = *horizontal* line; standard deviation = *triangle above* and *below* horizontal line; single values = circle)

to that of the live human; only $t_s$ was shorter in the cadavers (on the average, approx. 1–3 h). The considerable interindividual scatter in the studies on cadavers, like those in live humans, was impressive.

The question of whether this scatter may be dependent on other individual-specific factors, therefore, also arises. Further investigations should determine

**Table 4.** Site dependecy of kinetic data of epidermic cells from live humans; survey of the literature

| Location | Authors | LI (%) | $t_s$ (h) | $t_{pot}$ (h) |
|---|---|---|---|---|
| Arm | Pullmann et al. [18] | 2.9 | 8.0 | 276 |
| Upper arm | Pullmann [16] | 2.8 ± 0.1 | 7.2 ± 0.8 | 296 ± 121 |
| Upper arm-flexor side | Pullmann and Schumacher [19] | 3.3 ± 0.9 | 7.8 ± 0.4 | 25 ± 79 |
| Lower arm | Lachapelle [11] | 2.0 ± 5.0 | | |
| | Lachapelle and Gillman [12] | 2.2 (1.9–2.6) | | |
| Lower arm-flexor side | Camplejohn et al. [3] | 5.5 | | |
| Elbow | Galosi et al. [5] | 5.8 ± 2.5 | 9.0 ± 2.7 | 184 ± 91 |
| Leg | Pullmann et al. [18] | 4.4 | 7.8 | 195 |
| Abdomen | Allegra and De Panfilis [1] | 3.8 ± 0.8 | 7.6 ± 1.1 | 206 ± 51 |
| | Pullmann et al. [17] | 2.4 ± 0.16 | 6.6 ± 0.5 | 282 ± 96 |
| | Pullmann et al. [18] | 2.3 | 6.3 | 274 |
| | Pullmann [16] | 2.8 ± 0.1 | 7.2 ± 0.8 | 296 ± 121 |
| | Christophers and Schaumlöffel [4] | 3.8 | | |
| Dorsum | Braun-Falco et al. [2] | 4.5 | | |
| | Hell and Maibach [7] | 2.5 ± 1.2 | | |
| Buttock | Pullmann et al. [18] | 1.0 | 5.6 | 560 |
| Prepuce | Christophers and Schaumlöffel [4] | 8.6 ± 0.9 | 5.8 ± 0.3 | 142–164 |

if this scatter can be reduced by the selection of case material according to sex, age, and site. This question is also of particular interest because proliferation-kinetic data can be obtained from live humans only in exceptional cases and, consequently, little or no information is available. Taking into consideration that in cadavers $t_s$ shortened with increasing PMI, at least *relative* difference could be established even for this complex.

To the best of our knowledge, no studies on the sex-dependent proliferation of epidermic cells are available. There are also no systematic investigations on age dependency; the findings of a few authors who investigated live humans in certain age groups, however, are comparable. Camplejohn et al. [3] examined skin from 28 persons between 23 and 30 years of age and, after in vivo injection, found a LI of 5.5% (range: 4.1%–7.6%). Lachapelle and Gillman [12], who investigated six test persons between 45 and 58 years of age, reported a LI of 2.2% (range: 1.9%–2.6%) with in vivo labeling and a LI of 2.3% (range: 2.0%–2.6%) with in vitro labeling. Allegra and DePanfilis [1], who studied six test persons between 62 and 85 years of age using in vivo labeling, reported a LI of 3.79% ± 0.75%, a $t_s$ of 7.58 ± 1.22 h, and a $t_{pot}$ of 206.67 ± 50.92 h. As this comparison clearly shows, no important systematic differences exist.

Finally, the question of site-related differences of proliferation-kinetic data arises. The findings of various investigators have been compiled in Table 4 ac-

cording to their topographic allocation. This compilation shows that, with the exception of the prepuce, most DNA-synthesizing cells are found at the arm, particularly the elbow (LI, 5.8%), whereas the LI at other sites are, for the most part, identical (LI, 2.3%–3.3%). The table, however, does not take methodologic differences into consideration, i.e., in vivo or in vitro labeling, differing number of cases, varying dosages of radioactive marker and exposure times. Comparison of uniform findings is possible only with the study by Pullmann et al. [18]; these authors, however, investigated only four cases by in vitro incubation: one test person for each biopsy site (abdomen, buttock, arm, leg). They reported the highest LI and the shortest $t_{pot}$ in skin taken from the leg.

Our observations based on a comparatively large sample showed the highest LI, the shortest $t_{pot}$, and the largest epidermal diameter at the elbow. Our findings, therefore, are compatible with some of the other researchers listed in Table 4 (cp. [5]; see also [1, 4, 17–19]).

Our study did not consider the debated circadian rhythms of epidermic generation periods since they, as in the studies by Pullmann et al. ([17]. see also [6]), could not be corroborated in our few single tests.

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