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Cytokinetics of Epidermic Cells in Skin from Human Cadavers

I. Dependency on the Postmortal Interval*

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Summary. Punch biopsies taken from the extensor side of the thigh of 35 human cadavers were incubated in vitro with radioactively labeled thymidine (^{3}H-thymidine = ^{3}H-TdR, ^{14}C-thymidine = ^{14}C-TdR) to determine whether a relationship exists between changes in the proliferative activity of the skin and the postmortal interval after irreversible cardiac arrest. The cadavers were stored at 4°C. Cadavers with indeterminable time of death or presence of intoxication, drug therapy with cytostaic agents, or a skin disease were excluded from the study. Single, double, or multiple biopsies were performed on the same cadaver; single labeling with ^{3}H-TdR was done in all cases; double labeling with ^{3}H-TdR and ^{14}C-TdR in selected cases.

No relevant changes in the labeling index (mean, $2.39 \pm 1.03\%$) were demonstrable within the examined postmortal interval of 77.75 h. The DNA synthesis time (t_s) was, on the average, 4.75 ± 1.44 h; a certain relationship to the postmortal interval existed since t_s declined with increasing storage time. The potential doubling time (t_{pot}) decreased accordingly, beginning with 181.7 h (mean storage time, 29.9 h) and ending with 137.7 h (mean storage time, 41.7 h). No statistically relevant differences, however, were established at the 1% level. Whereas both labeling index and t_{pot} during the early postmortal interval are comparable with observations in live humans, t_s was relatively short as compared to that for the epidermis of live humans.

Key word: Time of death, cytokinetics of epidermic cells

Zusammenfassung. In vitro-Inkubation von Hautstanzen 35 menschlicher Leichen mit radioaktiv-markiertem Thymidin (^{3}H-Thymidin = ^{3}H-TdR; ^{14}C-Thymidin = ^{14}C-TdR) erfolgten unter der Frage der Stabilität der proli-

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ferativen Aktivität der Haut während des postmortalen Intervalles. Die Lagerung der Leichen erfolgte bei 4°C. Es wurden Einfachbiopsien, Doppelbiopsien und Mehrfachbiopsien an der selben Leiche durchgeführt; es erfolgte regelmäßig eine Einfachmarkierung (^{3}H-TdR) sowie in einer Reihe von Fällen auch eine Doppelmarkierung (^{3}H-TdR und ^{14}C-TdR).

Es wurde festgestellt, daß innerhalb des Untersuchungsintervalles von 77,75 h post mortem keine wesentlichen Änderungen des Markierungsindexes nachweisbar wurden; er lag im Mittel bei 2,39 ± 1,03%. Die DNS-Synthese-Zeit lag im Mittel bei 4,75 ± 1,44 h, wobei eine gewisse Abhängigkeit vom postmortalen Intervall insofern bestand, als sie mit zunehmender Lagerzeit der Leichen abnahm. Die potentielle Verdoppelungszeit betrug bei einer mittleren Lagerzeit von 29,9 h 181,3 h, während sie bei einer mittleren Lagerzeit von 41,7 h 137,7 h betrug. Statistisch relevante Unterschiede bestanden jedoch nicht. Während sowohl Markierungsindex als auch potentielle Verdoppelungszeit während des frühen postmortalen Intervalls mit Beobachtungen an lebenden Menschen vergleichbar sind, war die Sythese-Zeit bei Vergleich mit identischen Befunden lebender Menschen extrem kurz.

Schlüsselwörter: Epidermiszellen, Zytokinetik – Todeszeitbestimmung, Zytokinetik epidermaler Zellen

Introduction

Evidence for postmortal proliferation of epidermic cells, as for other cells of the human body, has been demonstrated only in a pilot study. Schellmann and Schell [25] determined the proportion of thymidine-incorporating cells in the skin of the rat; they reported a correlation between postmortal interval and proportion of thymidine-incorporating cells. In 1981, Schellmann [24] examined the proportion of thymidine-incorporating cells in the skin and oral mucosa of human cadavers and, likewise, observed a relationship between proportion of thymidine-containing cells and postmortal interval in the eight cadavers studied. Oehmichen and Zilles [17] found postmortal DNA synthesis in different organs from human cadavers and, additionally, demonstrated the presence of RNA synthesis.

Since no systematic investigations or quantitative results are available that can be compared with findings in the organs of live humans the labeling index (LI), DNA synthesis time (t_s) and potential doubling time (t_{pot}) of the basal cells in the skin from human cadavers stored for different periods of time were determined.

Material and Methods

Cadavers. A total of 35 cadavers were examined. Personal and investigatory data on the cadavers were provided by the police, family physican, and family members. Selection of the cadavers was based on the following criteria:

1. Determination of time of death within ± 2.2 h;
2. Absence of skin diseases;
3. Exclusion of intoxication or treatment with cytostatic agents.

The individual personal data are presented in Table 1. The postmortal interval ranged between 4.5 and 77.75 h; the age, between months and 88 years.

All cadavers received at our department were stored at approximately 4°C. An autopsy was made in some of the cases; the cause of death, as far as known, is listed in Table 2.

The skin specimen was taken with a biopsy punch (diameter: 3 mm). All biopsies (single, double, multiple) were taken from the extensor side of the thigh at different intervals after irreversible cardiac arrest. Approximately 80% of the biopsies were removed between 8:00 am and 4:00 pm.

Incubation. The in vitro labeling was made according to Helpap and Maurer [10 cp. 11, 12, 22] with the addition of ^{3}H-TdR (specific activity, 5 Ci/mmol; Amersham Buchler, Braunschweig, FRG) at a concentration of 20 µCi/ml. Some of the biopsy cylinders were additionally incubated in a second solution containing ^{14}C-TdR (specific activity, 54 mCi/mmol; Amersham Buchler, Braunschweig, FRG) at a concentration of 1 µCi/ml.

All specimens were incubated immediately after biopsy in Eppendorf caps at 37°C under 2.2 atm O_2.

The incubation time was 60 min. The skin specimens were washed 3 times in Basal Medium Eagle (BME; Serva Feinbiochemica, Heidelberg, FRG) after each incubation and then fixed in 4% paraformaldehyde with added 2'desoxythymidine (Serva Feinbiochemica) at a concentration of 1 mg/ml.

Histological and Autoradiographic Preparation. The paraplast-embedded biopsy cylinders were cut into 5 µm-thick serial sections. After the autoradiographic process, single-labeled sections were stained by Nuclear Fast Red. Double-labeled sections were stained by the Feulgen method prior to autoradiography.

Table 1. Age and sex of examined cadavers

Age (years)				*N*	Sex	
Age classes	Mean age ($\bar{x}$) (s)	Min	Max		f	m
< 15	6.4 ± 6.2	0.58	15	6	2	6
20–69	45.0 ± 14.3	20	66	15	8	7
> 70	78.7 ± 5.3	73	88	14	7	7

Table 2. Causes of death in examined cadavers

Causes of death	*N*
Acute cardiac death	9
Acute traumatic injury	7
Hanging	3
Intracerebral hemorrhage	1
Asphyxia	2
Protracted shock	1
Unclarified acute death	12

Autoradiographic processing was performed by the dipping method using Kodak NTB (single labeling) or Ilford K2 (double labeling – for the method: cp. Schultze [26]). The slides were exposed for 21 days in light-tight boxes.

Quantitative Evaluation. Only every third serial section was evaluated to avoid counting the same cell twice. The LI was determinded by counting 1,000 basal cells per section and calculating the percentage of labeled cells. Hair follicles and excretory ducts of sweat glands were not evaluated. T_s was determined by double labeling and claculated accordings to Schultze [26]; cp. [27]:

$$\frac{t_s}{\Delta t} = \frac{\text{Number of all } ^{14}\text{C-labeled nuclei (with and without } ^{3}\text{H-label)}}{\text{Number of purely } ^{3}\text{H-labeled nuclei}}$$

(Δt = time between ^{3}H- and ^{14}C-TdR injection)

T_{pot} was calculated according to Maurer and Schultze [16]:

$$t_{pot} = \frac{t_s}{LI}$$

The data were analyzed statistically by Student's *t*-test; a difference at the 1% level was considered significant [23].

Results

Labeling Index and Postmortal Interval

Single Biopsies. The labeling indices in cadavers with different storage intervals are presented in Table 3. In spite of the different intervals, the mean LI ranged between 19.8‰ and 30.6‰ with a broad scatter of approximately ± 10‰. The mean LI independent of the postmortal interval, was 23.9‰.

Double biopsies. Changes in the proliferative activity during the postmortal interval were evaluated by taking a second biopsy from the same cadaver (ten cases). These changes are graphically presented in the lower part of Fig. 1. Virtually no relevant change in the LI occurred during intervals ranging from 10 to 65 h. The mean LI was 27.1‰ (± 7.6‰) for the first biopsy and 28.0‰ ± 9.1‰ for the second biopsy, the mean time interval being 19.9 (± 6.3) h (range: 6.4–

Table 3. Mean labeling index (‰) in relation to postmortal interval

Postmortal interval (h)	*N*	LI (‰)	
		$\bar{x}$	s (±)
< 10	5	30.6	12.2
11–20	16	19.8	9.6
21–30	14	26.1	9.2
	35	23.9	10.3

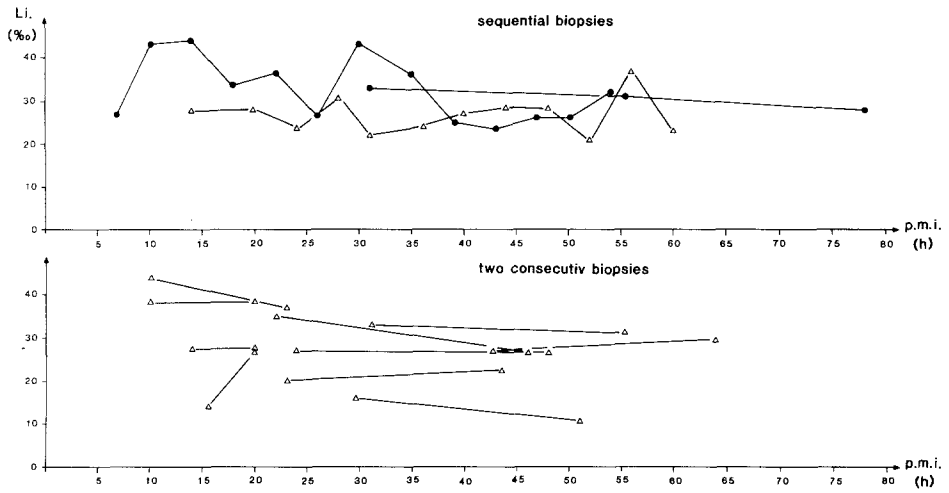

Fig. 1. Relation between labeling index and postmortal interval in multiple biopsies (three cases – *upper part* and in double biopsies (ten cases – *lower part*) from extensor side to thigh. No definite tendency toward postmortal interval-dependent changes are discernible

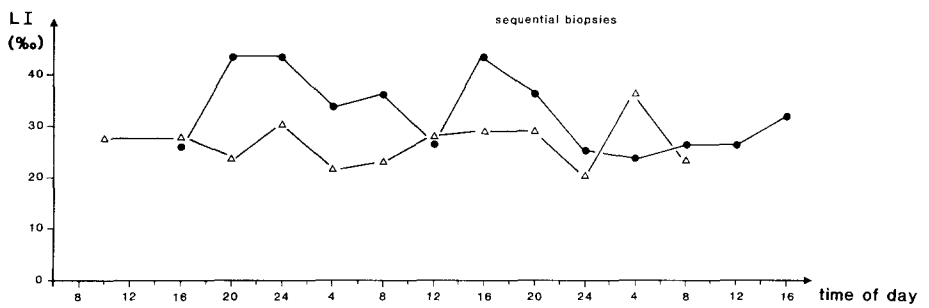

Fig. 2. Multiple biopsies from extensor side of thigh in two cadavers. Demonstration of same values. (cp. *upper part* of Fig. 1) in relation to time of day: classification according to biorhythmic variations was not evident

24 h). Even when only the nine double biopsies taken at similar intervals (20–24 h; mean: 22.7 ± 1.4 h) are considered, the relevancy of the differences is essentially the same (27.0‰ ± 6.0‰ or 26.75‰ ± 9.3‰).

Multiple Biopsies. Multiple determinations were carried out in three cases to obtain information on the course and, if possible, the biorhythm. The single values in relation to the postmortal interval are presented in the upper part of Fig. 1 and to the time of day (two of the three cases) in Fig. 2. No relevant changes in the LI were established during the investigated interval of 5–60 h during which biopsies were taken at 4-h intervals. A relationship between LI and circadian rhythm was not demonstrable in the two examined cases.

T_s and Postmortal Interval

In ten cases, t_s was determined by double labeling; two biopsies were taken at different postmortal intervals in five of these cases. The values, including the corresponding postmortal intervals, are listed in Table 4. According to the values for the first biopsy there is no distinct trend in the investigated postmortal interval of 10–64 h. The mean t_s was 4.75 ± 1.44 h; single values ranged between 2.73 h (minimal) with a postmortal interval of 12 h, and 7.78 h (maximal) with a postmortal interval of 10 h.

When, however, the change of the t_s in the same cadaver, as demonstrated by double biopsies, is considered (Table 4) t_s decreased in almost all cases during the postmortal interval. The mean t_s at the first biopsy (mean PMI: 29.9 ± 17.6 h) was 4.75 ± 1.44 h, whereas it was 3.2 ± 1.1 h at the second biopsy (mean PMI: 41.7 ± 12.4 h). The mean difference in the postmortal intervals was 19.9 ± 5.8 h.

T_{pot} and Postmortal Interval

The relation between calculated t_{pot} and PMI are presented in Table 5. No relationship between t_{pot} and PMI is evident in column 2 (t_{pot}/h). The mean t_{pot} was 181.3 ± 57.2 h. If the changes of t_{pot} are followed in the cases with two biopsies (cp. columns 2 and 4 in Table 5), t_{pot}, with one exception, decreased during the postmortal interval. The mean t_{pot} which was determined, on the average, 19.9 h later, was now 137.7 ± 40.4 h.

Table 4. DNA Synthesis time and postmortal interval determined by single or double biopsies

	1st biopsy		2nd biopsy		Postmortal interval between 1st and 2nd biopsies (h)
	Postmortal interval (h)	t_s (h)	Postmortal interval (h)	t_s (h)	
	10	7.78	20	3.76	10
	12	2.73			
	22	3.47	46	4.67	24
	22.5	4.13			
	23.75	5.67	43.5	3.09	19.75
	24	4.18	48	2.71	24
	29.5	4.33	51	1.75	21.5
	35	5.67			
	56.5	4.0			
	64	5.56			
$\bar{x}$	29.9	4.75	41.7	3.2	19.9
s	17.6	1.44	12.4	1.1	5.8
N	10	10	5	5	5

Table 5. Potential doubling time and postmortal interval determined by single or double biopsies

	1st biopsy		2nd biopsy		Postmortal interval between 1st and 2nd biopsies (h)
	Postmortal interval (h)	t_{pot} (h)	Postmortal interval (h)	t_{pot} (h)	
	10.0	199.5	20	96.4	10.0
	12.0	124.1			
	22.0	99.1	46	179.6	24.0
	22.5	179.6			
	22.75	283.5	43.5	140.5	19.75
	24.0	149.3	48.0	96.8	24.0
	29.5	254.7	51.0	175.0	21.5
	35.0	138.3			
	56.5	200.0			
	64.0	185.3			
$\bar{x}$	29.9	181.3	41.7	137.7	19.9
s	17.6	57.2	12.4	40.4	5.8
N	10	10	5	5	5

Discussion

Our results are presented in Table 6. Whereas virtually no change in the labeling index was found in our investigated material with increasing postmortal interval (maximal storage time, 77.75 h; storage temperature, 4°C), t_s was extremely short. Moreover t_s and t_{pot} tended to change, i.e., they decreased during increasing postmortal interval; since, however, these differences are not significant, no definitive conclusions can be drawn. The short t_s as well the decrease of t_s and t_{pot} during the PMI may be explained by the absence of a steady state of the proliferatory system, i.e., not all cells proliferate [26]. The question arises as to whether the calculated t_s and t_{pot} correspond to a real DNA synthesis time or a real potential doubling time. The double labeling method for determining t_s might be influenced by the increasing PMI leading to a change of the cell flux through the cycle. Since the calculated t_{pot} depends on t_s, t_{pot} tends to decrease as does t_s.

The extent to which findings in cadavers obtained by systematic investigation are comparable with results in live humans is an important question. The data obtained in the available in vitro studies on live humans are presented in Table 7. As the number of examined cases is usually low, LI, t_s and t_{pot} differ considerably. The labeling index values ranged between 1.37% ± 0.44% and 8.6% ± 0.9% [4]. T_s, however, was between 5.83 ± 0.33 h [4] and 10.3 ± 0.6 h [7], and the t_{pot} between 142 h [4] and 282 ± 9.6 h [19]. We, therefore, conclude that the labeling index obtained in cadavers is comparable with that in live humans. The same also holds true for t_{pot} within the first 30 h post mortem; but t_s was always lower than that observed in live humans.

Table 6. Cytokinetic data for epidermis of human cadavers

Kinetic criteria	Findings $\bar{x}$ s	Mean postmortal interval (h)
LI (%)	2.71 ± 0.76	21.8 ± 11.1
	2.80 ± 0.91	41.7 ± 14.7
t_s (h)	4.75 ± 1.44	29.9 ± 17.6
	3.2 ± 1.10	41.7 ± 12.4
t_{pot} (h)	181.3 ± 57.2	29.9 ± 17.6
	137.7 ± 40.4	41.7 ± 12.4

One fundamental question is whether the numerical results are based on a true incorporation of the applied labeled thymidine by DNA or whether this is a paraphenomenon. Due to the preceding Feulgen staining of several sections, nearly all proteins, with the exception of the nucleic acid-containing chromatin, were leached out of the section; a regular synthesis process must be assumed since nuclear labeling remained unchanged.

Preliminary investigations [1] have shown that cells of other organs also display a certain proliferative activity postmortally; in most cases, however, this activity stops within 20–30 h post mortem. The enduring proliferative activity of the skin, therefore, represents an exception, which has been substantiated by morphological and biochemical findings. Using light microscopy, Braun-Falco and Winter [3] investigated epidermal changes during autolysis and detected no light microscopic signs of autolysis in skin stored for as long as 5 days at 4°C. The relationship between oxygen consumption of the skin and postmortal interval was investigated by three research teams [1, 5, 15]: All observed a decrease of oxygen consumption with increasing postmortal interval. Berg et al. [1], in particular, pointed out the rapid decrease in oxygen consumption in relation to storage temperature. Lindner [15] and Berg et al. [1] investigated the incorporation of ^{35}S sulfate in subepidermal connective tissue and observed incorporation up to 3 days post mortem in tissue stored at low temperatures.

The findings from Schellmann [24] can be interpreted similarly; he investigated proliferative activity by taking punch biopsies at 2- to 4-h intervals from three cadavers stored at 20°C and four cadavers stored at 4°C. Proliferative activity was demonstrable up to 36 h post mortem in the cadavers stored at 20°C and between 48 and 100 h post mortem in those stored at 4°C. Whereas Schellmann's demonstration of proliferating cells in the skin was substantiated by our findings, we were not able to confirm his observation of a linear decrease in ^{3}H-TdR-labeled cells or the biorhythm. A detailed comparison between his quantitative findings and our results is not possible since he counted the number of labeled cells per longitudinal unit of skin.

On the basis of our observations, we conclude that, particularly at low temperatures, the proliferative activity of the skin, due to its direct contact with atmospheric oxygen, continues for a longer period of time after irreversible cardiac arrest.

Table 7. Cytokinetics of basal cells in skin from live humans after in vitro labeling procedure according to data presented in the literature

Author(s)	*N*	Age	Site	LI (%)	t_s (h)	t_{pot} (h)
Braun-Falco et al. [2]	19	16–87	Dorsum	4.5		
Christophers and Schaumlöffel [4]	5	(?)	Abdomen Prepuce	3.75 8.6	5.83 ± 0.33	142–166
Lachapelle [13]	15	(?)	Lower arm	2–5		
Lachapelle and Gillman [14]	6	45–58	Lower arm	2.2 (1.9–2.6)		
Heenen and Galand [8]	6	(Adults)	(?)	4.8 (3.8–5.5)	10 (9.7–10.4)	213 (177–279)
Hell and Maibach [9]	6	(?)	Dorsum	1.37 ± 0.44		
Heenen et al. [7]	3	(?)	5.8 ± 0.6	10.3 ± 0.6	184 ± 26	
Steigleder et al. [28]	6	22–53	Upper arm	2.03	6.6	316 (267–383)
Pullman et al. [19]	3	30–54	Upper abdomen	2.4 ± 0.16	6.6 ± 0.5	282 ± 9.6
Pullman et al. [20]	4	(?)	Leg	4.0	7.8	195
Pullman and Schumacher [21]	5	(?)	Upper arm	2.9 ± 0.8	6.7 ± 1.6	220 ± 67
Pullman [18]	17	(?)	Upper arm	2.8 ± 0.1	7.2 ± 0.8	296 ± 121
Galosi et al. [6]	7	(?)	Elbow	5.8 ± 2.5	9.0 ± 2.7	184 ± 91

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