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Experimental Studies on the Kinetics of Early Postmortem Decay of the Lymphocyte Membrane*

Remarks About Its Application to Forensic Hemogenetics and to Cornea Transplantation

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Summary. Tests on human and animal cadavers were carried out to investigate the early postmortem decay of the lymphocyte membrane.

HLA-typing of cool-kept human cadavers can be performed within the first 36 h after death. Hereby, a possibility is obtained to determine HLA-ABC antigens of a deceased for hemogenetic or cornea transplantation purposes. The data obtained from animal experiments give evidence that the decay of the lymphocyte membrane follows a function of the Verhult's type. Using this function, it is possible to estimate the rate constant as a function of temperature.

Key words: HLA-typing of cadavers – Estimation of the time of death, HLA-typing – Cornea transplantation, HLA-typing

Zusammenfassung. An menschlichen wie tierischen Leichen wurden Untersuchungen zur Kinetik des frühen postmortalen Zerfalls der Lymphozytenmembran durchgeführt. Es ergab sich, daß direkte HLA-Typisierungen an Leichen innerhalb von 36 h nach dem Tode möglich sind, sofern die Umgebungstemperaturen unter 10° C gelegen haben. Man erhält hierdurch die Möglichkeit, HLA-ABC Antigene zu forensischen Zwecken und für Cornea-Transplantationen zu bestimmen. Die tierexperimentellen Studien lieferten den Hinweis, daß der Zerfall der Lymphozyten nach den Gesetzmäßigkeiten der Verhultschen Gleichung verläuft. Bei Gebrauch dieser Funktion ist eine Möglichkeit gegeben, die Zerfallskonstante als Funktion der Temperatur zu messen, womit eine Todeszeit-Bestimmung versucht werden kann.

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Schlüsselwörter: HLA-Typisierung von Leichen – Todeszeitbestimmung, HLA-Typisierung – Corneatransplantation, HLA-Typisierung

The purpose of this paper is to report a simple method for the rapid and direct determination of HLA-ABC antigens of human cadavers as well as the early postmortem (temperature-dependent) decay of the lymphocyte membrane.

Subjects and Techniques

Human Cadavers

Up to now, 24 deceased were provided for our examinations by chance of legal autopsy. Two or three hours after death, the cadavers were brought into the cooling chamber of our institute. At the beginning of the examinations, the probands had been dead for about 3–62 h. Death was due to different causes.

Rat Cadavers

After killing 48 young rats, half of them were kept at 4° C, the other half at 21° C. At particular intervals, thymuses of three rats were ectomized simultaneously.

Samples of *peripheral blood* were taken from *human* cadavers by puncture of the femoral vein.

Inguinal lymph nodes and *spleen pulp* of *human* cadavers as well as *thymuses* of young *rats* were prepared and perfused by using a solution consisting of RPMI 1640, 25 mM Hepes + L-glutamine.

Lymphocytes were separated by the density gradient method. The obtained cells were washed twice with (Ca^{++} & Mg^{++} free) Hank's solution. Tests were performed with 2,000 cells/µl.

HLA-typing of the human lymphocytes was carried out by means of the NIH standard technique [7], but the time of incubation with complement was diminished to 45 min to avoid remarkable cross-reactions. The *background-rates* of the *thymus-lymphocytes* were counted after adding eosin (5% in Hank's solution) and fixation with formaldehyde.

Results

Experimental Results

Animal Experiments. The results of our experiments with (thymus-lymphocytes of) rat cadavers are summarized in Table 1. Rat cadavers kept at 21° C did not carry any viable lymphocyte in their thymuses 8 h after death, whereas it took 96 h to observe the same result in cadavers kept at 4° C. Figures 1 and 2 illustrate the mode of background increase in the animal experiments.

Experiences with Human Cadavers. The most intriguing problem in HLA-typing of cadavers by means of the NIH technique is the large amount of dead cells (= background). From our experiments it can be learnt that the lymphocyte suspensions obtained by lymph node perfusions (= LNL, *L*ymph *N*ode *L*ymphocytes) meet the blood groupers' demands best if compared to cell preparations of spleen pulp or peripheral blood [3, 6]. Therefore, we refrain from demonstrating the results of these mistrials and exclusively discuss the findings with LNL in this paper. For it is not informative to report on HLA-phenotypes of 24 deceased, we want to draw

Table 1

Rat cadavers kept at 21° C		Rat cadavers kept at 4° C	
Time of death [h]	Background [%]	Time of death [h]	Background [%]
0	0.1	3	0.1
1	0.1	6	0.1
2	1	24	10
3	3	30	25
4	10	48	50
5	40	54	65
6	75	72	85
7	90	78	90
8	100	96	100

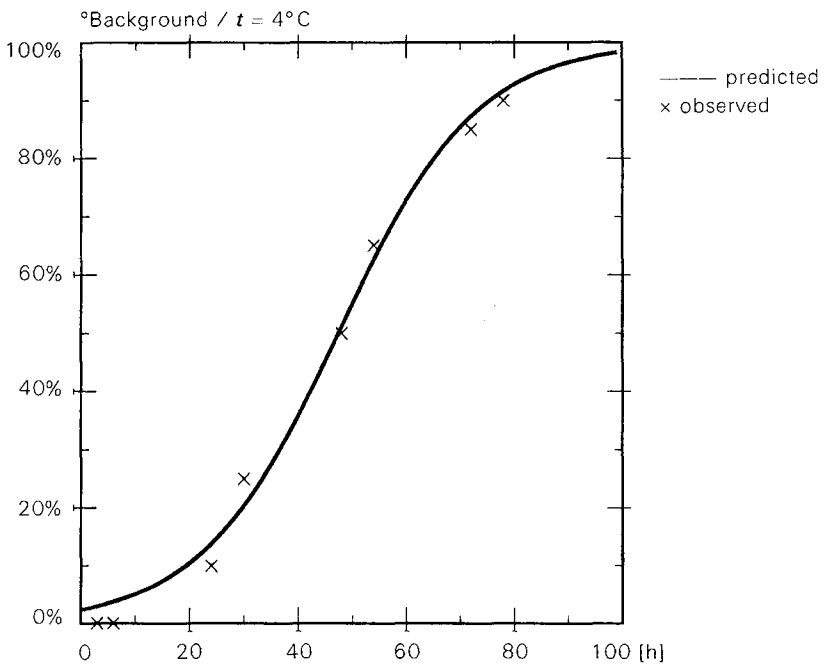

Fig. 1. Time course of background of rat lymphocytes at 4° C. The data are fitted to Eq. 9

attention to the particular background rates. Figure 3 illustrates the increase of the background in relation to the time interval between death and the testing.

Figure 4 is demonstrating the backgroud rates of those cases, when just a single ectomy could be performed. A slight increase of the background occurs within the first 24 h after death, whereas within the following hours, the increase becomes more significant. Within the 1st day, the backgroud hardly exceeds 10%, and this

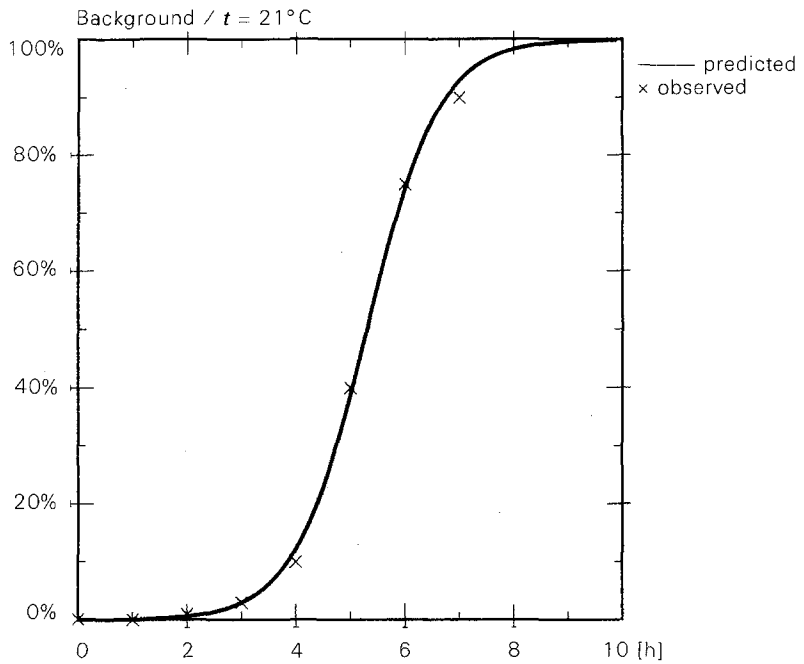

Fig. 2. Time course of background of rat lymphocytes at 21° C. The data are fitted to Eq. 9

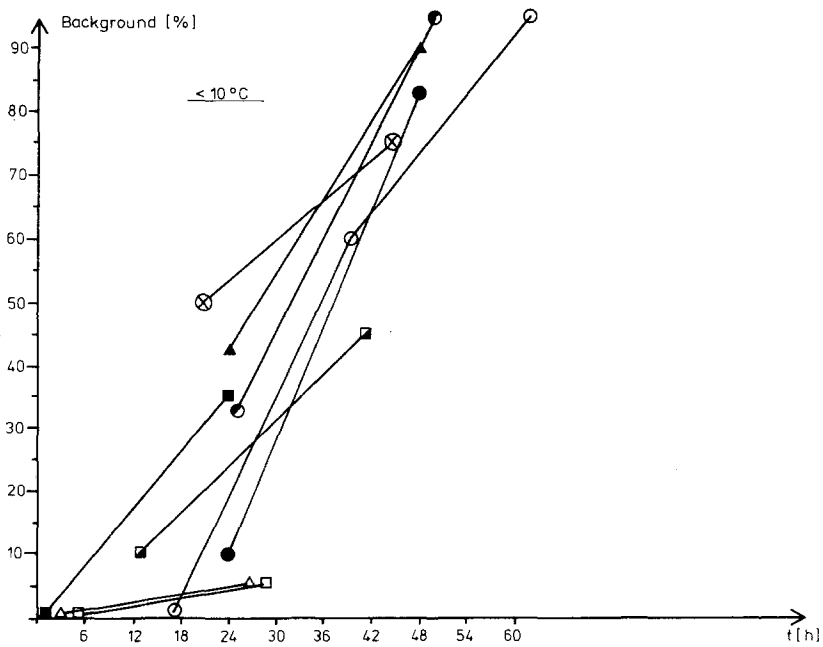

Fig. 3. Background increase of lymphocytes of human cadavers, when lymph nodectomy could be repeated

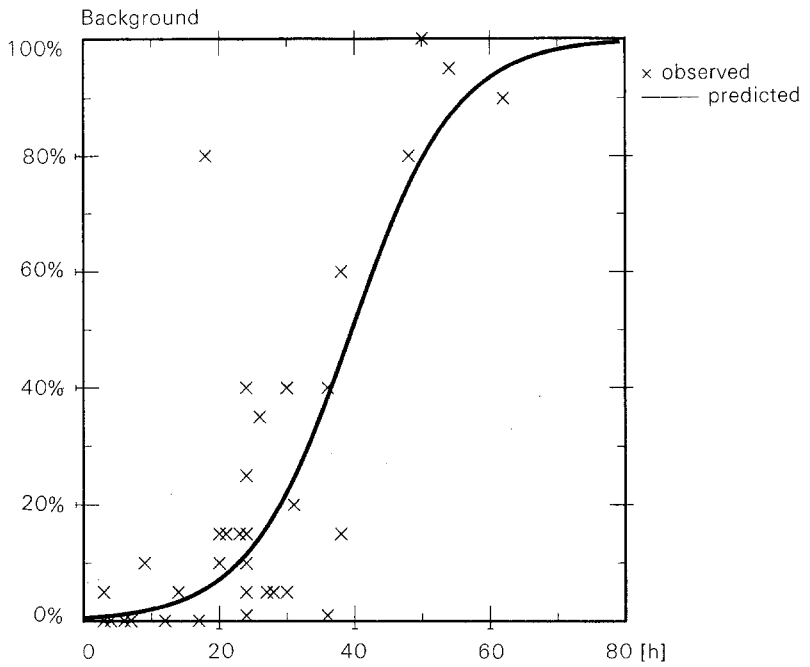

Fig. 4. Background rates of lymphocytes of human cadavers in random cases obtained by single ectomies

usually allows a reliable HLA-typing. Apart from this, we would emphasize that HLA-typing of (cool-kept) cadavers is generally possible within the first 36 h after death, when the background does not exceed a value of around 40%.

Mathematical Model. The data of the animal experiments allow to assess the kinetics of the decay of lymphocytes. In case of a monoexponential decay, the number of cells alive at a time t is given by

$$n_a(t) = n_a(O)e^{-kt}. \qquad \text{Eq. 1}$$

The number of dead cells as a function of time is given by

$$n_d(t) = n_d(O)(1 - e^{-kt}). \qquad \text{Eq. 2}$$

As it can be seen by inspection of the data, Eq. 2 does not apply to the type of curves encountered in our experiments. According to Eq. 2 the increase of dead cells is maximal at $t = 0$. The experimental curves exhibit a different behavior: only after a time lag, which is dependent on temperature, a marked increase in dead cells occurs. Thus, a monoexponential decay must be ruled out. Furthermore, one has to assume that the kinetics involved are non-linear.

Since the experimental curves resemble much the type of curves encountered in autocatalytic reactions, the following model is considered, which is based on the assumptions:

(i) a dead cell produces some agent y, which in turn increases the rate of decay; (ii) the amount of y is proportional to the number of dead cells, e.g., $y = a\, n_d(t)$. The kinetic equations are given by

$$\frac{d}{dt} n_a = -\mathrm{k}\, \mathrm{n}_a \mathrm{n}_d \qquad \text{Eq. 3}$$

$$\frac{d}{dt} n_d = \quad k n_a n_d \qquad \text{Eq. 4}$$

with $n_a + n_d = n_{ao} = \text{const.}$ Eq. 5

and the initial conditions

$$n_a(O) = n_{ao} \text{ and } n_d(O) = n_{do} > 0\,. \qquad \text{Eq. 6}$$

By use of Eq. 5 Eq. 3 becomes

$$\frac{d}{dt} n_a = -\frac{d}{dt} n_d = -k\, n_a (n_{ao} - n_a)\,. \qquad \text{Eq. 7}$$

Eq. 7 has the solution

$$n_d(t) = \frac{k\, n_{ao}}{k + \left(\frac{k\, n_{ao}}{n_{do}} - k\right) e^{-k\, n_{ao} t}}\,. \qquad \text{Eq. 8}^{*}$$

This is Verhult's logistic curve, which is a mathematical description of a S-shaped curve with the asymptotic value

$$n_d(\infty) = n_{ao}\,.$$

If one takes n_{ao} as 100%, Eq. 8 can be rewritten in terms of "percent" of dead cells, $\bar{n}_d$:

$$\bar{n}_d(t) = \frac{100}{1 + \left(\frac{100}{\bar{n}_{do}} - 1\right) e^{-k' t}} \qquad \text{Eq. 9}$$

where $k' = n_{ao} k$.

Eq. 9 proved to be a suitable mathematical description of the experimental curves and was used for estimation of the parameters k' and $\bar{n}_{do}$.

The estimated parameters are summarized in Table 2.

Table 2

T [°C]	k' [l/h]	$\bar{n}_{do}$ [%]
[a]	0.13	0.55
4	0.078	2.38
21	1.51	0.03

[a] Human cadavers

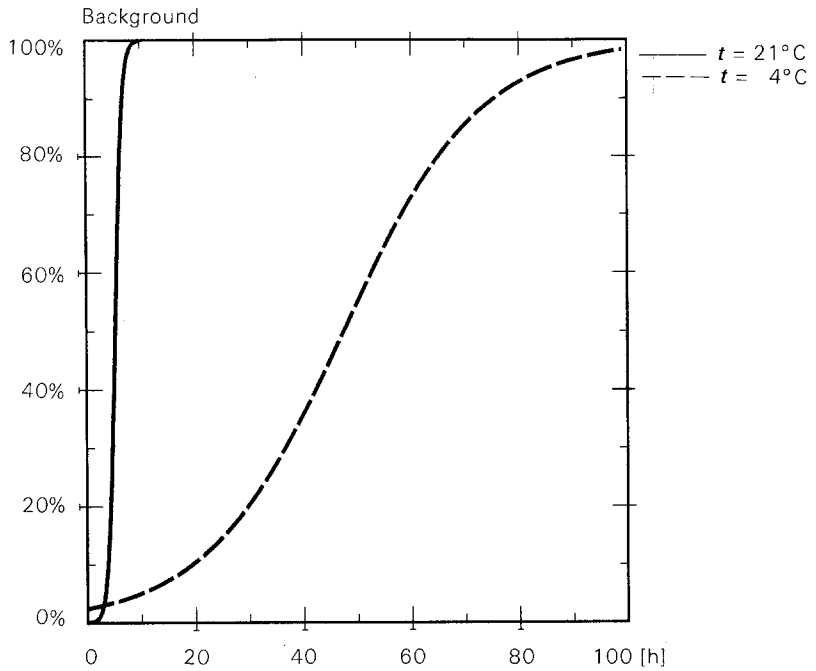

Fig. 5. Time course of background of rat lymphocytes at both 4° and 21° C. The data points are omitted (Figs. 1 and 2)

The data obtained from human cadavers are not homogeneous with respect to temperature and exhibit great variances. Nevertheless, the data are fitted best by a Verhult type of curve, when compared to a linear regression (Fig. 4).

The data reveal a remarkable temperature dependence of the reaction constant k': at 4° C, the decay proceeds at a rate which differs by a factor of approximately 20 from the rate obtained at 21° C. This result is illustrated in Fig. 5, where both curves are plotted in one diagram.

Discussion and Conclusion

Several time-consuming tests exist (e.g., fluorochromasia, microabsorptions) for HLA-typing which can be used if a high percentage of dead lymphocytes is present in the negative control. It is obvious that those tests are restricted to specially equipped laboratories and adequate mainly for research purposes.

It is known that direct postmortem HLA-typing can reliably be performed if LNL were taken as typing material; last but not least, because the LNL suspension usually is highly pure. The particular disadvantages of lymphocyte suspensions from other sources were demonstrated [3, 6]. From our experiments it can be learnt that human cadavers kept at temperatures below 10° C (indicating a minor rate of decay) allow the performing of HLA typing within the first 36 h after death. These results suggest that it is feasible to apply the introduced method to forensic

hemogenetics [4] or to cornea transplantations. Preliminary experiences with HLA-matched corneal grafts in high-risk cases made by Gibbs et al. [2], Kok-van Alphen and Völker-Dieben [5], Völker-Dieben et al. [8, 9], Ehlers and Kissmeyer-Nielsen [1] reveal evidence for the usefulness of acknowledging histocompatibility antigens in such kind of ophthalmologic effort.

On the other hand, further experiments (at constant temperature conditions) are necessary to assess the kinetic law of the decay of lymphocytes. By this it will be possible to determine the rate of decay as a function of temperature. This information has not merely a theoretic aspect, but may find application in medicolegal practice (e.g., to determine the time of death).

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