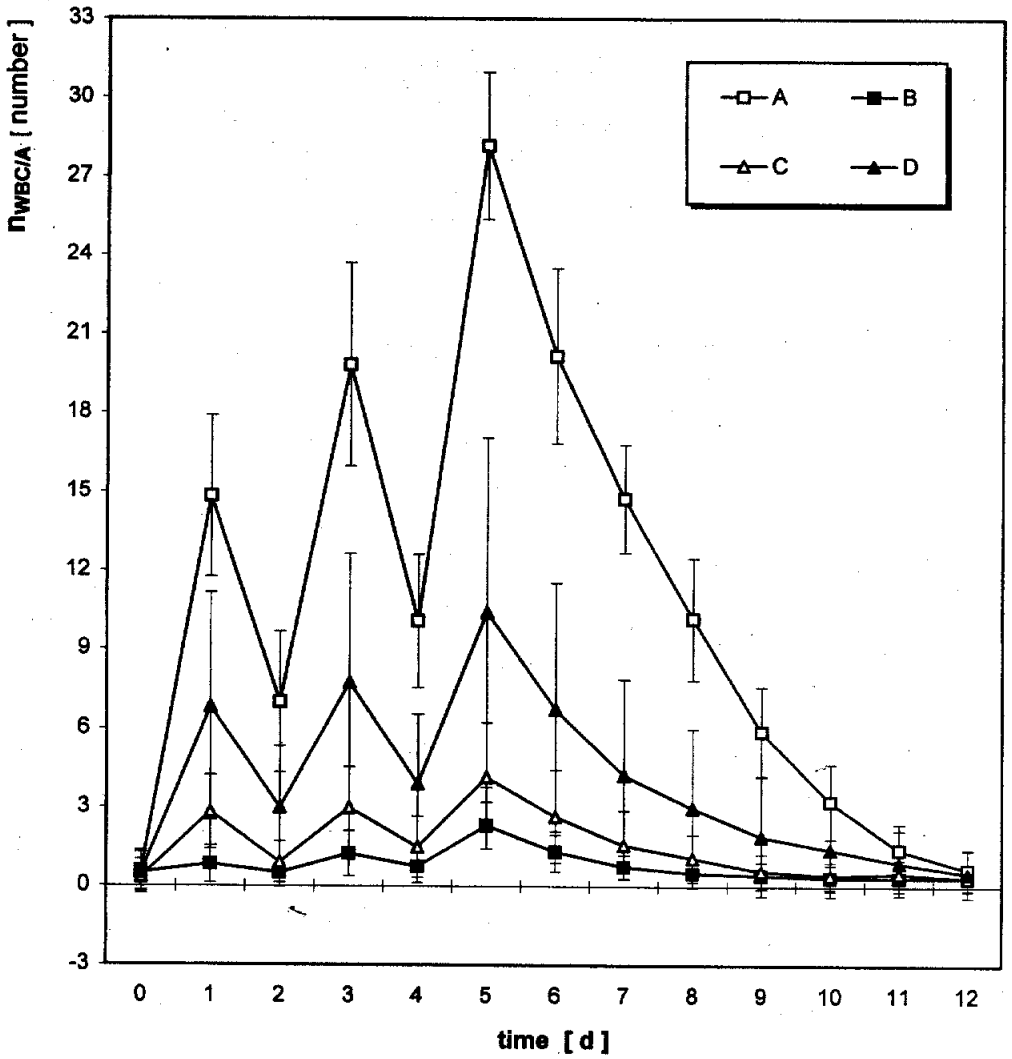


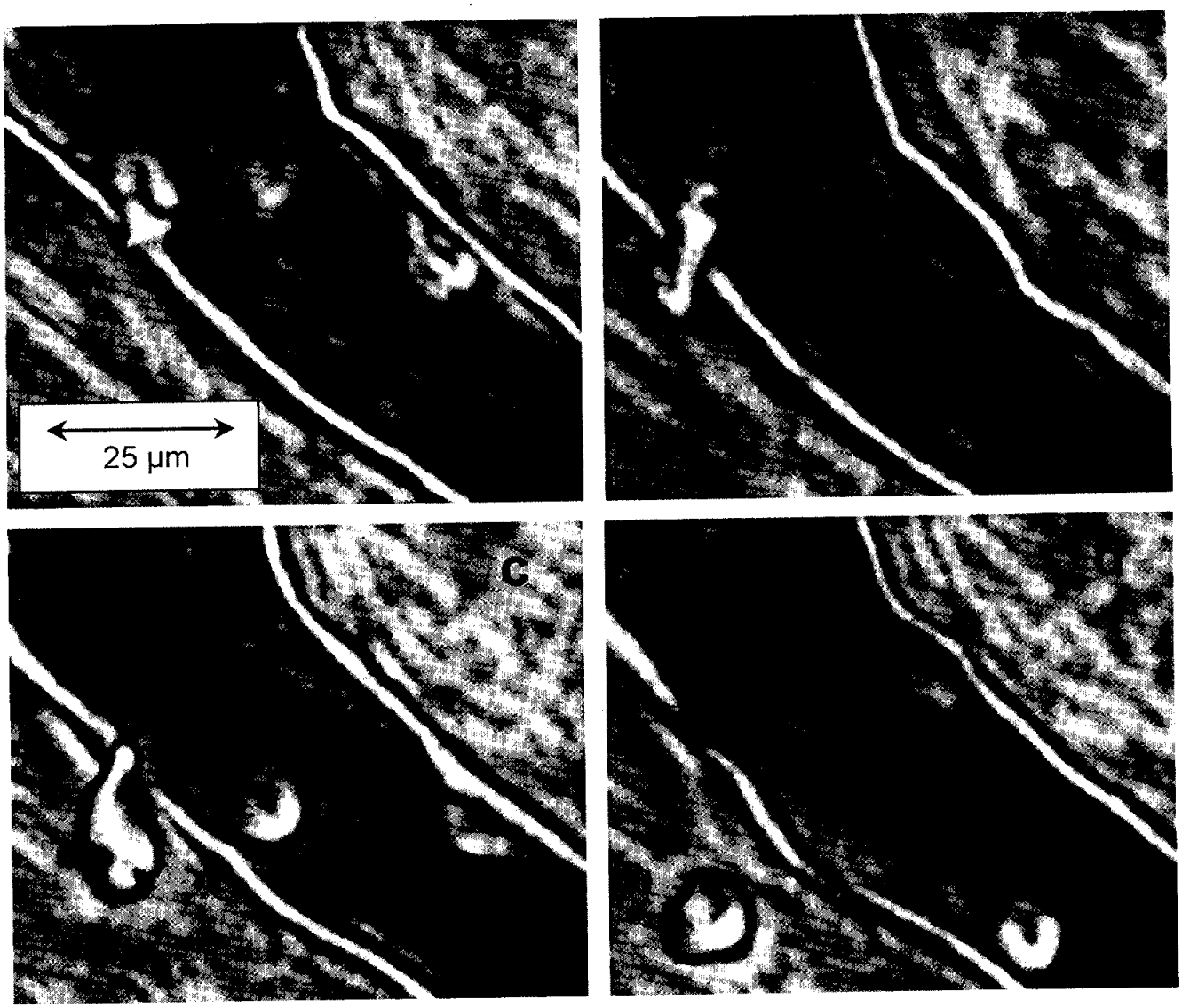

Figure 6. *"Number of adhering white blood cells on a defined venular surface nWBC/A" (mean values and standard deviations) from various target tissues: A, derma / injection point; B, derma / thorax; C, gingiva; D, rectum. Abscissa: time (days). Ordinate: number per definition surface.*

**Results and Discussion**

After each mistletoe extract injection there was an increase in rectal temperature, most pronounced and statistically significant when compared to the initial values after the final injection ( 1.0 mg per mL ). Systolic blood pressure and heart rate were upregulated accordingly. This, obviously was a regulatory reaction of the blood pressure correlating to the basal body temperature of the test subjects. Thus, temperature and blood pressure regulation behaved principally similarly to particular stages of mild fever and suggested a reaction of the total organism to the "stimulus" of the mistletoe extract injection. Erythematous skin changes were observed at the mistletoe extract injection sites. Accordingly, changes in the cutaneous and subcutaneous microcirculation were determined in the target tissues.

Using vital microscopy, Figures 2a and 2b representatively show the distribution of blood cells in the subcutaneous microvascular network at the injection point on day 0 ( before the mistletoe extract administration ) and on day 5 ( after the third injection ). On day 5 a larger number of blood cell perfused nodal points ( junctions in the network ) and an increase of the venular stream was determined. This suggests an optimized distribution of blood in the microvascular network with physiologically advantageous pressure gradients between the arterioles and the venules. Apparently the local regular breadth of the microcirculation was ( temporarily ) expanded. This effect may also influence the body's own resistance mechanisms, especially the immunological behavior of white blood cells.

Figures 3a – 3d show the adhesion of white blood cells towards the venule endothelium in the subcutis within the




Figures 7a – 7d. *Transmigration of a white blood cell through a venular wall into the interstitium of a volunteer after mistletoe extract injection day 5 in the rectal target tissue. Vital microscopy, mf 1/8000 s. The image sequence shows a time interval of 8 s from a to d the procedure of the passage of a white blood cell through the venular endothelium from adhesion to presence in the extravasal space.*

local reaction on days 0, 1, 3 and 5. With each injection, increasingly more white cells could be found in the plasma edge of the venular microvessel. The cells 'roll-off' of the endothelium to adhere. This indicates advantageous conditions for the initial steps of the immune reaction.

Thus, after administration of mistletoe extract, an improved function of the dermal microcirculation and an immunologically advantageous behavior of white blood cells were determined at the injection point. These changes obviously represented local immunological skin reactions. The characteristic changes observed resembled those that occur in the "infection phase" of (undisturbed) wound healing (30). Similar changes can be found during the healing of an *Ulcus cruris* after previous restoration of the physiological regulation of the (local) microcirculation. (29) The question arose as to whether these characteristic changes are limited local reactions without therapeutic benefit, or whether they affect the entire organism. Figures 4, 5, 6, and 8, summarize the data which were obtained simultaneously in various areas of target tissues of the derma and intestine.

Figure 4 shows comparable effects on the distribution of the blood cells within the microvascular networks (characteristic $n_{NP}$) in any investigated area of the target tissues. Significant changes were temporary, increasing with each injection but fading away almost to the levels of the initial values about one week after the last injection. The

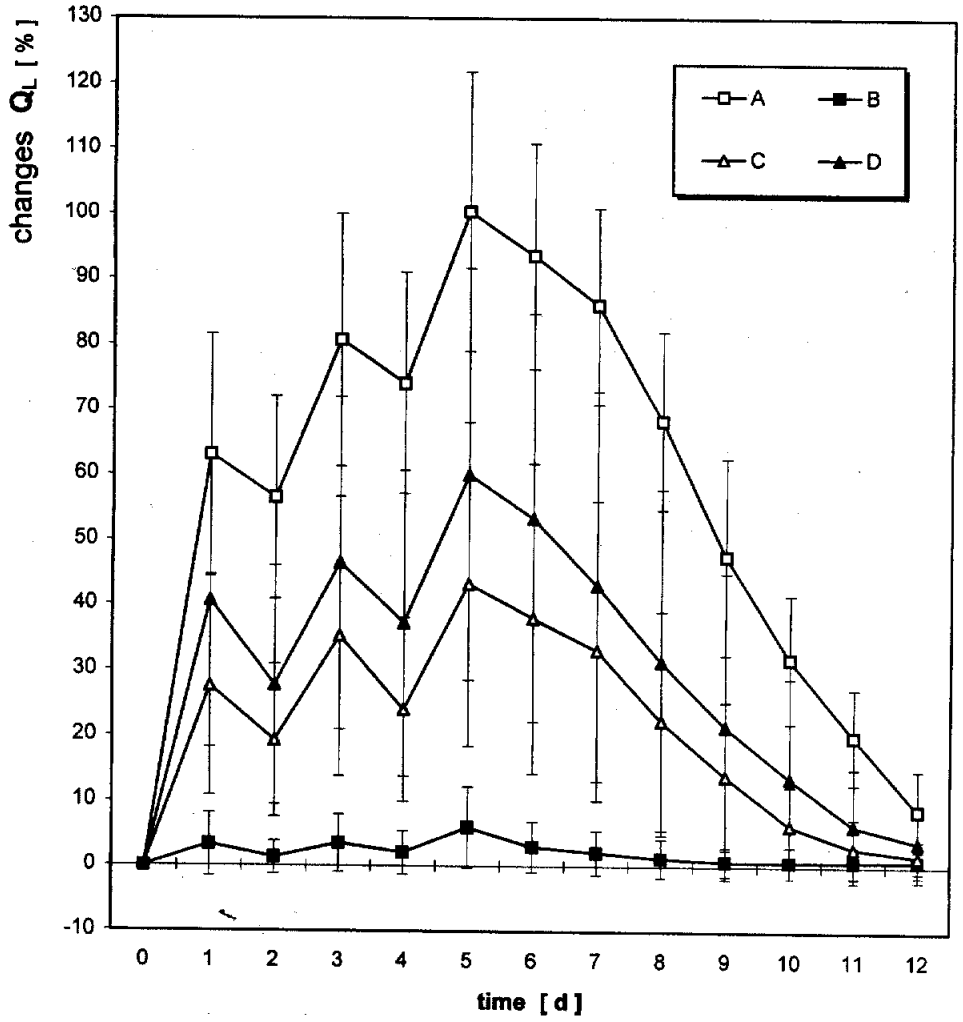



Figure 8. *"Streaming flow of the initial lymph $Q_L$" (mean values and standard deviations) from the various target tissues: A, derma / injection point; B, derma / thorax; C, gingiva; D, rectum. Abscissa: time (days). Ordinate: percentage in changes as compared to the initial values.*

venular streaming ($Q_{ven}$) showed a corresponding behavior in all target tissues investigated, as shown in Figure 5. This improved distribution was not a microhemodynamic disadvantage since, in connection with an increased cell stream, it may be regarded as physiologically advantageous. This characteristic behavior was obviously the result of a significant change in the local flow characteristic of the blood in the microcirculation within the target tissues, besides the change in the size of the dynamic functions of the microcirculation. By means of the rheological characteristic "tube hematocrit $Hk_t$" (minor) local hemodilutions were found after the mistletoe extract injections. This steady increase may be interpreted as a further sign of the (temporarily) improved functional condition of the microcirculation after mistletoe extract administration. The greatest changes in local hematocrit appeared on day 5 and achieved microhemodynamically relevant values of ~10% in comparison with initial values.

Improvements of the functional conditions of the microcirculation in different immunologically active target tissues after mistletoe extract administration have an influence on the realization of transportation phenomena for tissue nutrition. However, yet another aspect is of greater importance: the influence of function of the microcirculation on the immunological behavior of white blood cells. Figure 6 shows that the improved function of the microcirculation after mistletoe extract administration also induced a significantly increased adhesion tendency of the white blood cells (characteristic $n_{WBC/A}$). This phenomenon was not limited to the local reaction, but could be determined similarly in other immunologically active tissues.

It is known that the function of the microcirculation is an

important determinant for the first steps of immunological reactions (29,30). To become immunologically active, white blood cells must first be transported by the blood stream to defined tissues. Then they have to pass the microvascular networks to guarantee an adequate distribution. They enrich in the plasma of "larger" microvessels. On their way to be further functionally active they 'roll-off' the endothelium, adhere, and finally pass through gaps in the microvascular wall into the interstitium. An unimpeded course of roll-off, adhesion and transmigration phenomena assumes particular microhemodynamic conditions. Corresponding with the behavior of the characteristic $n_{WBC/A}$, a significantly increased transmigration behavior of the white blood cells was found (characteristic $n_{WBC/A}$), which was shown not only at the point of the local reaction, but also in other target tissues investigated. An example of these findings is given in Figures 7a – 7d, showing the process of transmigration by vital microscopic image sequence. The data of the characteristic "streaming flow of the initial lymph $Q_L$" are given in Figure 8. The characteristic and biometrically significant differences indicate that the immune reaction in the area of the initial lymph flow was advantageously influenced in all target tissues examined

The results of the local concentrations of the immunologic communication protein (Interleukin-1) secreted from leukocytes are a semi-metabolic reference to the investigated functional and behavioral characteristics. Interleukin-1 reflection spectrometrical measurements showed that a stimulation of the immune regulation occurs after mistletoe extract administration in the dermal as well as the intestinal target tissues. This stimulation, however, was not limited as a local reaction.

In conclusion, after administration of standardized mistletoe extract, significant changes in the function of the microcirculation and in (immunological) characteristics of the white blood cells appeared, which increased with the drug injections and declined a few days after the final injection. These characteristic changes, were imposed not only locally at the point of injection, but also in other tissues or organs. Thus, the concept of "local reaction" concerning skin effects at the point of mistletoe extract administration is confusing, since the characteristic changes are not limited to the point of injection but rather affect the entire system and only become "apparent" as a local appearance. Independently of the differing extent of the characteristic changes in various target tissues, the simultaneous and similarly directed characteristic behavior in various target tissues is biologically relevant.

Mistletoe extract administration induced a temporary immunomodulation in the healthy volunteers investigated, which can be considered as a strengthening of the natural resistance mechanisms. The type and extent of the changes observed are comparable to those of infectious processes under *physiological* conditions.

Further investigations have to clarify the questions whether, how and to what extent the immunomodulating effect of standardized mistletoe extract (Iscador®) can be demonstrated during pathological conditions (*e.g.* cancer, infectious diseases). The results so far available can be regarded as promising.

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*Received June 19, 2001*
*Accepted August 30, 2001*