

SCANNING ELECTRON MICROSCOPY OF THE INITIAL LYMPHATICS OF THE SKIN AFTER USE OF THE INDIRECT APPLICATION TECHNIQUE WITH GLUTARALDEHYDE AND MERCOX® AS COMPARED TO CLINICAL FINDINGS

PART I: THE NOMENCLATURE AND MICROTOPOGRAPHY OF THE INITIAL LYMPHATICS

B.I. Wenzel-Hora, D. Berens von Rautenfeld, A. Majewski, D. Lubach

Department of Radiology, Clinical Research, Schering AG, Berlin (BIW-H), Zentrum Anatomie, Medizinische Hochschule Hannover (DBvR), Department of Radiology, Oststadt-Hospital, Hannover (AM), Department of Dermatology, Medizinische Hochschule, Hannover (DL), Federal Republic of Germany

ABSTRACT

Certain phenomena in the region of the initial lymphatics during clinical examination (indirect lymphography and microlymphography)—namely build-up and course and filling and diffusion—have still to be elucidated. The glutaraldehyde and MERCOX® methods were used to simulate indirect application conditions in clinical procedures in skin specimens (human and pig) which were then examined by scanning electron microscopy. The results presented include novel findings on the microtopography of these lymph vessels and the typology of filling refluxes which are of importance when indirect imaging techniques are employed. Various speeds of application were used and the function of the opening apparatus with previously undescribed structures (endothelial bridges and connective tissue trabeculae) was taken into consideration. Recommendations aimed at standardizing the nomenclature of parietal structures and sections of the lymphatics have been incorporated in the report.

Recent years have seen the development of new clinical and morphological examination techniques which allow systematic evaluation not only of the collectors, intranodal lymph sinuses and lymphatic trunks, but also of the initial lymphatics (initial lymph sinuses, lymph capillaries and precollectors)(1). Using the new techniques, the initial lymphatics (IL) can be filled from the interstitial connective-tissue space with various injection boluses applied indirectly (i.e., interstitially). The new methods of demonstration are based on the principle of indirect application employed by many workers at the beginning of the 19th century for the anatomical demonstration of collectors (2). The initial lymphatics in the skin of man were first evaluated radiologically and vital-microscopically by means of simultaneous indirect lymphography (3-5) and microlymphography (6-9). Appropriate scanning electron microscopic (SEM) studies of the IL in various organs of different animal species have been conducted after use of the indirect MERCOX® and indirect glutaraldehyde (GA)

methods (10-13). There are so far no corresponding studies of the IL in the skin of man, although they appear necessary to clarify the microtopography and fine structure of the dermal lymphatics in relation to the rate of application (14,15). To correct this situation, the present study therefore deals primarily with the effects of filling, transport and diffusion phenomena in the region of the IL of the skin of man after administration of glutaraldehyde, MERCOX® and IOTASUL. Our studies have not yet progressed far enough to take account of the categorization of age-related or pathological changes of the dermal lymphatics (16-18), since the available study material does not lend itself to statistical analysis.

Since SEM examinations can only be performed in human biopsy material, corresponding experimental findings in the intact skin of anesthetized pigs were compared. The pig was chosen as the experimental animal because its integumentum commune is structurally and physiologically comparable with that of man in many respects (19).

MATERIALS AND METHODS

The examination material for scanning electron microscopy (SEM) consisted of biopsies from 20 patients with malignant melanomas taken from macroscopically unchanged areas of skin at a "safe" distance of 5cm from the tumor. The lymphatics of the scalp formed the focal point of this study. Out of 20 specimens of skin from the head region, 10 samples each were prepared for SEM. MERCOX® or the fixation fluid glutaraldehyde was usually applied no later than 10 minutes after excision of the skin specimens.

In 30 pigs 12-14 weeks old, fluids were applied under anesthesia into the skin on the head and other parts of the body. The indirect glutaraldehyde (GA) method was performed with 4% GA to which 2% Berlin blue had been added to stain the lymphatics. The interstitial injections were administered intradermally with 2ml disposable syringes (Braun, Melsungen, FRG) via a Venofix (Braun, Melsungen, FRG) and an electronically regulated infusion pump (Precidor 5003, G. Heinemann, Schwäbusch Gmünd,

FRG) at application rates of 0.003, 0.03, and 0.3ml/min. With this approach, the initial lymphatics became dilated and were fixed in this state. The material was prepared in the usual way for SEM and, in some instances, also for transmission electron microscopy (TEM).

The *indirect MERCOX® (MX) method* was performed manually with methylmethacrylate MERCOX® (Japan Vilene Co., Ltd., Tokyo). After a setting time of at least one hour, the tissue block was transferred to 25% potash lye or caustic soda. The specimens were rinsed daily under running water and placed in fresh lye until maceration of the soft tissue was complete. Preparation and examination of the specimens were as described above.

In previous experimental projects conducted under application conditions identical to those employed in the indirect GA method, IOTASUL was injected using the *indirect contrast medium (CM) method*. Extensive clinical and preclinical x-ray material (man, monkey, dog, pig) was available for the microtopographical categorization of the course of the dermal lymphatics.

Nomenclature of the initial lymphatics

There are two principal reasons why the anatomical nomenclature (NOMINA ANATOMICA, 1983, NA, and NOMINA HISTOLOGICA, 1983, NH, Williams and Wilkens, Baltimore/London, 5th Edition, 1983) of lymphatic vessels and structures requires radical revision: numerous new microtopographical and electron microscopic findings which are absent in the NA and NH, and the vast difference between the anatomical and clinical names for lymphatics. The Latin nomenclature newly proposed by us is signified by the initials NT (new termini) in contradistinction to the familiar initials of NA or NH.

Morphological microtopography of the IL

Fundamental knowledge about the microtopography of the lymphatics in the corium had to be obtained before attempting to categorize the different sections of the

lymphatics in indirect lymphography and microlymphography. The corium (dermis) contains two interwoven networks of initial lymph sinuses (*sinus lymphatici initiales*, NT; *vasa lymphocapillaria*, NH) and precollectors (*vasa lymphatica precollectoria*, NT). These initial lymphatics (*vasa lymphatica initialia*, NT) display considerable regional differences in the density of the two vascular section, i.e., different mesh sizes in different areas of the skin of man and pig. Species-specific peculiarities are, however, less frequent.

RESULTS

Fig. 1 is a schematic but scale representation of the microtopography of the lymphatics in the skin of the head as shown by our studies. The depicted course of the lymphatics is the same for different areas of the head (ear, temples, forehead, cheeks). Unlike other regions of the integument, the two networks of initial lymphatics in the skin of the head are particularly dense. The mesh size of the two

vascular sections is, in general, smaller around hairs and cutaneous glands than in hairline regions.

The network of *initial lymph sinuses* (diameter about $50\mu\text{m}$) forms mesh-like (mesh size: $200\text{--}500\mu\text{m}$) and blind-ending segments in the corium. This network does not display any consistent pattern. Short, blind-ending segments of sinuses occur in an irregular framework in the region of both subepidermal and deeper-lying vascular networks in the corium. Blind-ending and mesh-like segments of sinuses come to within $20\mu\text{m}$ of the basal membrane of the epidermis. Lymph sinuses are also present around hairs and cutaneous glands in the connective tissue coating close to the outer margin of epidermal complexes.

The *precollectors* (mean diameter $100\mu\text{m}$)—the first postcapillary lymphatics equipped with valves—form a homogeneous net, the meshes of which display a diameter of more than $500\mu\text{m}$ but no blind-ending vascular segments. Unlike those of the collectors, the valves of the precollectors are barely de-

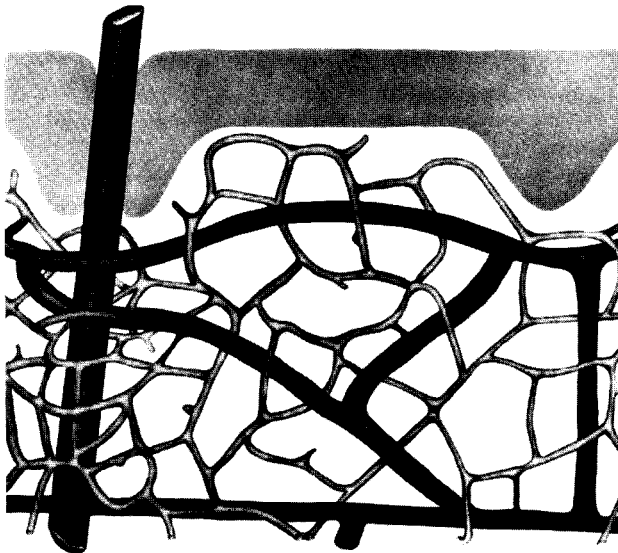


Fig. 1. Initial lymphatics of the skin of the head of man as a scale drawing. The narrow-meshed network of initial lymph sinuses shows blind-ending vascular segments which are developed not just in the region of the papillary body. The network of precollectors displays valves, larger vascular caliber and meshes. Only an end segment of the precollector network (at the bottom of the picture) descends into the subcutis (not shown). Note the narrow sections of the vascular networks of initial lymphatics around the hair follicle.

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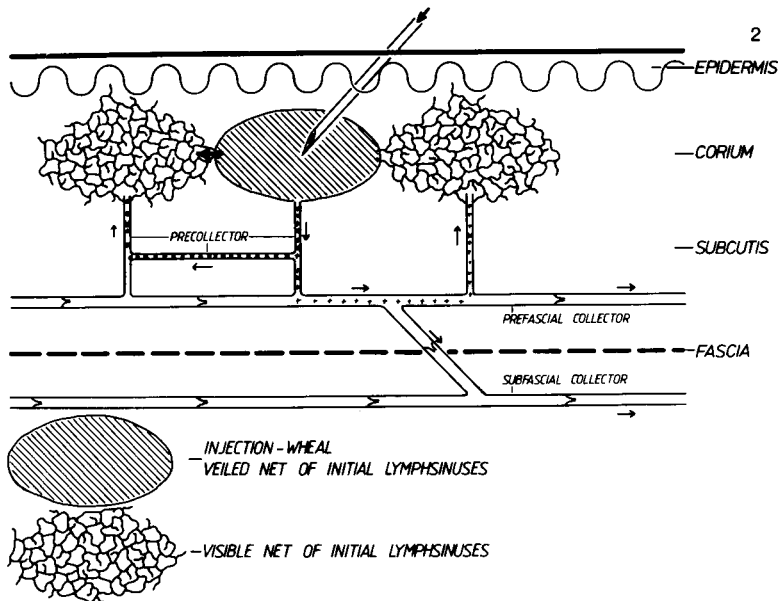


Fig. 2. Schematic representation of reflux phenomena after indirect application of fluids in the skin. Injection wheal (hatched) with intercapillary reflux (thick arrow); precollector reflux (...); collector reflux (+++).

tectable from the contours as dips between dilated vascular sections. Consequently, precollectors do not have the beaded appearance typical of collectors. Precollectors can come to within $50\mu\text{m}$ of the basal membrane of the epidermis. Relatively few drainage pathways exist from the precollectors to the collectors. The end sections of the precollectors considerably limit the filling capacity of the IL. The precollectors commonly open into collectors running parallel to the skin surface and epifascially (vasa lymphatica collectoria superff., NT), but rarely into subfascial collectors (vasa lymphatica collectoria proff., NT). In man, peripheral collectors display a diameter of about $200\mu\text{m}$.

Microtopography of the IL from a clinical standpoint

Both initial lymph sinuses and precollectors are documented by microlymphography and indirect lymphography. Each method al-

lows differentiation of the two sections of initial lymphatics on the basis of diameter (initial lymph sinus up to $70\mu\text{m}$, precollector from $70\mu\text{m}$), but not on the basis of shape because precollectors do not have a beaded appearance. However, the findings also show that epidermal tumors can invade lymph sinuses and precollectors over short interstitial distances of $20\mu\text{m}$ and $50\mu\text{m}$, respectively. In contrast, blood capillaries can be situated directly on the basal membrane of the epidermis. Moreover, the small mesh size ($300\text{-}500\mu\text{m}$) of the initial lymphatics in the skin of the head makes them more important during inflammation (e.g., in the clearance of inflammatory cells) than those in other areas of skin (e.g., on the trunk). The few afferent precollectors that open into subfascial collectors apparently act as an overflow system when the indirect application of fluid overfills the tributary region of epifascial collectors. Consequently, the indirect application of IOTASUL rarely leads to radiological demon-

stration of the deep (subfascial) system of collectors, since only small amounts of contrast medium reach the vasa collectoria proff.

Filling reflexes and application technique

The following definitions are required for an understanding of the filling reflexes (c.f. also Figs. 1-3):

1. The *lymphatic area* of skin consists of a precollector running obliquely (drainage pathway to a collector) with a network of initial lymph sinuses and draining precollectors. The networks of initial lymphatics of adjacent skin lymphatics cannot be structurally demarcated from one another.
2. *Intercapillary reflux*: Horizontal drainage of fluids via one or more lymphatic areas of skin within the initial lymph sinus (Fig. 2).
3. *Precollector reflux*: Drainage of fluids from initial lymph sinuses or a lymphatic area of skin via precollectors to the initial lymph sinuses of another lymphatic area (Fig. 2).
4. *Collector reflux*: Drainage pathway from collectors via precollectors into a network of initial lymph sinuses (Fig. 2).

Various reflux phenomena are common when high injection rates (0.3ml/min) are used for indirect application into the corium. However, reflux can also be induced by blocked drainage of diverse origin (see below). Reflux can be demonstrated radiologically after use of indirect lymphography with IOTASUL (Fig. 3) and morphologically with the indirect MERCOX® method. Our studies show that the drainage of indirectly applied fluids in the skin depends primarily on the filling capacity of the precollectors which open into a collector (Figs. 1-3). Thus, the lymphatic area of skin can take up only a limited amount of fluid from an initial sinus network. If the filling capacity of the precollectors is exceeded, intercapillary and, sometimes, precollector reflux may occur. No networks of lymph sinuses are seen in the injection wheal (MERCOX® or IOTASUL)(Fig. 3), but they are visible in the initial lymph sinuses of lymphatic areas of skin next to the wheal. The

spread of injection wheals is limited at injection sites by profuse connective tissue septa and high papillary bodies of the corium (e.g., the foot and hand). As a result, filling of initial lymph sinuses is accelerated. Collector reflexes occur in particular less frequently as a result of high infusion pressures in the area of application and more frequently as a result of blockages in the region of prenodal and nodal lymph drainage. In the case of precollector and collector reflux, valves in precollectors must be overcome in a retrograde direction.

Our experience with simultaneous indirect lymphography has taught us not to place several cannulas in one lymphatic area of skin but rather, if possible, to place them in neighboring areas to reduce the incidence of intercapillary reflux (Fig. 4). When this is done, only a single collector or a bundle of collectors usually fills with the injected fluid via several precollector tributaries. If, on the other hand, networks of initial lymph sinuses are to be demonstrated morphologically (MERCOX®) or radiographically (IOTASUL), then intercapillary reflexes next to the injection wheal are necessary for the evaluation of initial lymphatics (Fig. 3). This means that the injection cannulas should not be placed in neighboring lymphatic areas of skin if capillary reflexes are to be created around the injection cannula. The arrangement of the cannulas largely depends on the size of the lymphatic area of skin. Small areas with a dense pattern of initial lymphatics have a diameter of no more than 1.5cm. These are located not only in the skin of the head, but also in that of the hands and feet. Further measurements of lymphatic skin areas in different regions of human skin are required if the application technique is to be improved.

DISCUSSION

The present SEM studies of human skin biopsies are closely comparable with the findings in the initial integument of the pig and other animal species with the indirect GA method. The *morphological microtopography* of the initial lymphatics (IL) in the skin is a controversial subject. Disagreement also exists regarding the optimal nomenclature for the initial lymphatics (13,14,20,21). Two to

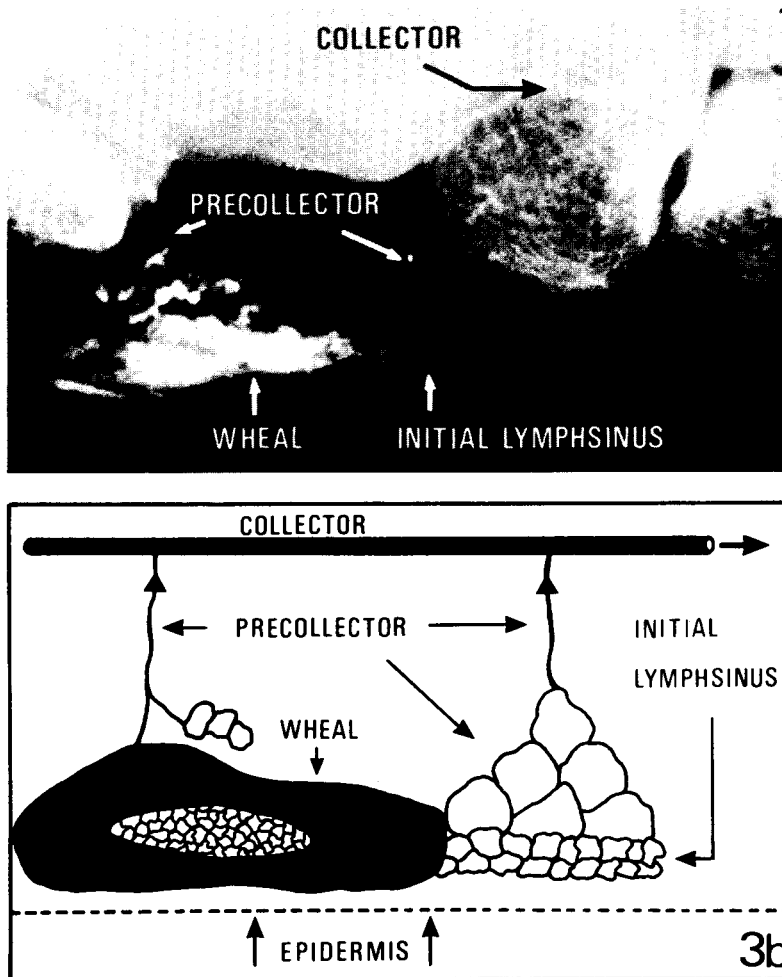


Fig. 3. Indirect IOTASUL lymphography a) in the skin of man (foot) with graphic reconstruction. b) Intercapillary reflux right next to an injection wheal. The network of initial lymph sinuses not recognizable in the wheal by lymphography has been inserted in the center of the outline. One precollector of each of two lymphatic areas of skin drains the contrast medium into a collector paralleling the skin surface.

three dermal networks of initial lymphatics are claimed (22-25). More recent reports describe a superficial network of initial lymph sinuses (ILS) and a deep—but still in the corium—network of precollectors (13,14). According to our studies in the skin of the head, the two networks are interwoven. As a result, both the ILS and the precollectors come to within $20\mu\text{m}$ and $50\mu\text{m}$, respectively,

of the basal membrane of the epidermis. From the present findings, not only lymph sinuses but also precollectors can be recognized by vital microscopy in microlymphography (6,7,9). In agreement with others, filled initial lymph sinuses display a maximum diameter of $70\mu\text{m}$ and an average diameter of $50\mu\text{m}$ (14,17,26-28), while precollectors display a diameter of more than $70\mu\text{m}$ with an average of $100\mu\text{m}$ (14).

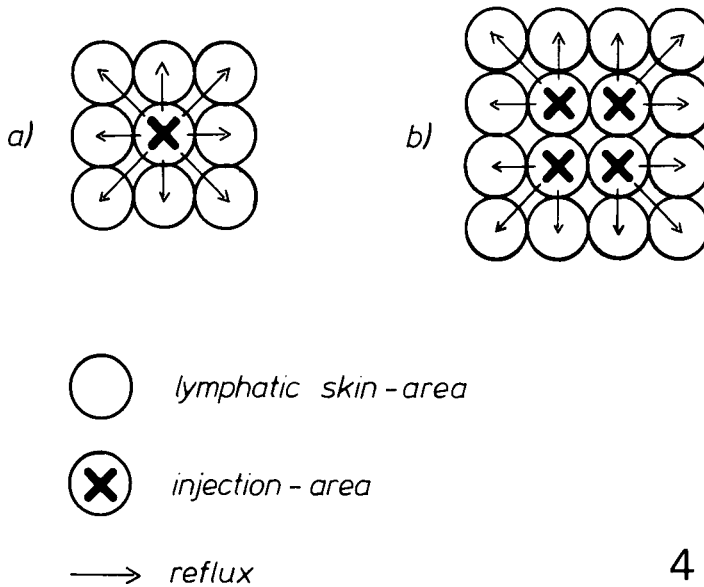


Fig. 4. Intercapillary refluxes (arrows) on indirect application of a fluid a) into a lymphatic area of skin and b) into 4 adjacent skin areas. The reflux rate is theoretically reduced by 50% in b).

The initial lymphatics with diameters of more than $70\mu\text{m}$ documented by Jäger/Bollinger (8) using microlymphography are therefore probably not initial lymph sinuses, but precollectors. Earlier data concerning the presence of valves in initial lymph sinuses are also contradictory. Using TEM, Daroczy (29) and Leu (30) claim to demonstrate valves even in initial lymph sinuses, whereas we interpret these luminal structures on the basis of our SEM studies as endothelial bridges (see below). Since valves cannot be recognized in the subepidermal precollector sections because of their structure either by microlymphography or by lymphography, the size (transverse diameter) of the vessels should also be used to decide proper classification. Only the afferent segments of the precollectors to the collectors contain valves (with valvular dilatations) which are also demonstrable using clinical methods of examination. This circumstance also explains why some authors have been unable to demonstrate valves morphologically in the lymphatics of the corium using the injection methods (22,25,31),

while others (14,32) were unable to demonstrate them histologically. Previous data also disagree as to the mesh size of initial lymphatics in the skin of man and its allocation to the two vascular sections. According to Ise-nring et al (7), Bollinger et al (1), and Kubik/Manestar (14), the mesh size of initial lymph sinuses is $320\text{-}640\mu\text{m}$ in different areas of skin of the fingertips. Our studies show that, in the skin of the head, smaller mesh sizes of $200\text{-}400\mu\text{m}$ are common, whereas precollector networks display a diameter of more than $500\mu\text{m}$. Based on our skin findings, the mesh diameter (of about $500\mu\text{m}$) reported by Jäger/Bollinger (8) using microlymphography are, therefore, precollector sections.

The lymphatic area of skin and the pre-collector refluxes play a crucial role in regard to the filling capacity of initial lymphatics using the indirect application technique. According to Kubik (33), the lymphatic area of skin comprises two networks of initial lymphatics (initial lymph sinuses and precollectors) which drain by an obliquely or vertically descending precollector into a col-

lector. On the basis of our studies, the diameters of the skin areas are maximally 1.5cm in the skin of the head, the soles of the feet, and the palms of the hands, and 3-4cm in the rest of the integument (33). If the application is made with an injection cannula in each of the neighboring lymphatic areas of skin (c.f., Fig. 4), the result is a relative reduction of intercapillary reflux phenomena to a theoretical 50%. As a result, fluid transport is directed towards the end sections of the precollectors. At the same time, care must be taken with the simultaneous indirect application that the filling capacity of the lymphatic areas of the skin is not exceeded; these areas form strip-like zones of skin (33), so that a bundle of collectors can be filled selectively via its skin zone. The filling reflux phenomena studied and defined by us

morphologically can also be recognized clinically (34,35) after instillation of dye into the skin for the diagnosis of functional disorders in the region of the initial lymphatics. Not only intercapillary, but also precollector refluxes occur frequently particularly after use of high injection rates (0.3ml/min), a technique used to visualize initial lymph sinuses outside the injection wheal. Intercapillary refluxes occur horizontally over larger areas of skin in human and animal fetuses and also in the immediate postfetal period (23,33,36), since the precollector network is still integrated in the network of initial lymph sinuses in the absence of a complete set of valves (B. v. Rautenfeld, personal observation). This observation suggests that the centrifugally directed valvulogenesis (32), is still not completed in the early postfetal period.

REFERENCES

See end of Part II.

B.I. Wenzel-Hora. M.D.
Department of Radiology
Clinical Research
Schering AG
Müllerstrasse 170-178
D-1000 Berlin 65