

## Collection and Physiological Measurements of Peripheral Lymph and Interstitial Fluid in Man

W.L. Olszewski

Dept. for Surgical Research and Transplantation, Medical Research Center, Polish Academy of Sciences, Warsaw, and Laboratory for Hematology and Lymphology, Norwegian Radium Hospital, Oslo

### Summary

Methods used for collection and measurement of physiological parameters of peripheral lymph and interstitial fluid in man have been described, basing on the experience of lymphological groups from Warsaw and Oslo and on data from the relevant literature. The review consists of description of methods for chronic cannulation of leg lymphatics, collection of lymph from skin lymphatic vessels, measurement of peripheral lymph flow, lymph hydrostatic and oncotic pressures, collection of interstitial fluid with the capsular, liquid-paraffin cavity, wick and skin window methods, interstitial fluid hydrostatic and oncotic pressure measurement.

### Introduction

Improved methods for investigating the lymphatic system and interstitial space in man extends our knowledge of those mechanisms regulating tissue transport of water, solutes, and proteins; local metabolic processes; and migration and homing of cells in non-lymphoid tissues. Many results of studies carried out in man confirm what was observed in animal models or inferred from animal experiments. There are, however, also a large number of original observations related specifically to human physiology or pathology, which could be made only because the exploratory methods were extended to man, e.g., observations of diurnal variations in protein concentration in leg lymph (8), protein concentration and output in leg lymph during physiological changes in capillary filtration (23), low and differentiated concentrations of various classes of immunoglobulins and complement proteins in lymph (24), lack of major differences in lymph composition between patients with lymphedema and normal subjects (25), enhanced interstitial fluid and lymph formation in long-term diabetes (18),

interstitial fluid pressure of skin of the limbs and subcutaneous tissue in man (29, 30), defected recirculation of lymphocytes through peripheral tissues in patients with chronic lymphatic leukemia (6). Results of these and other studies not mentioned here point to the necessity for the improvement and broader application of the exploratory methods for lymph and interstitial fluid in man.

In man, it is more convenient to sample and study lymph than the interstitial fluid, because the lymphatics are easily accessible and large volumes of lymph are available. The problem, however always exists as to whether lymph is representative for the interstitial fluid and, if so, to what extent. Recent studies (32) have largely solved this problem indicating that there are no significant differences between fluid protein level in, lymph and interstitial, at least not in the skin and subcutaneous tissue.

For collection and studies of human skin and subcutaneous tissue lymph and interstitial fluid, limbs seem to be most suitable. The material obtained originates from a rather homologous tissue, the influence of physical (capillary and venous pressure, external pressure, temperature), nervous and hormonal (nervous stimulation, hormones, drugs), and immune (infection, antigenic stimuli) factors on water and protein transport as well as cell migration can be studied. It should here be mentioned that collection and *in vivo* studies of lymph and interstitial fluid must be carried out under uniform and strictly defined conditions. Factors such as location of the tissue from which the fluid is derived in regard to heart level, metabolism, passive and active movements of the tissue ambient temperature and the temperature of the tissue the itself,

local and systemic nervous stimuli, drugs, water and electrolyte intake or output, and traumas; even the time of the day may considerably affect the volume and composition of lymph and interstitial fluid.

In the following sections, methods for collection and measurement of physiological parameters of peripheral lymph and interstitial fluid in man will be described which are based on the experience of groups from the Surgical Research Laboratory, (Polish Academy of Sciences), the Laboratory for Hematology and Lymphology (Norwegian Radium Hospital), and data taken from relevant literature.

### Collection of Limb Lymph

#### *Anatomy of the Lymphatics of the Leg*

Lymphatic trunks of the leg may be divided into a deep and superficial system. The latter consists of the medial and lateral groups. In the lower leg, the medial superficial group is composed of two to three collecting trunks. They cross the long saphenous vein at the level of the knee but never close to it (26) and terminate in the inguinal lymph nodes. The lateral superficial group consists of one to two trunks apposed to the short saphenous vein running toward the popliteal fossa. The superficial system drains lymph from the digital lymphatic plexuses, foot and calf skin, and subcutaneous tissue. The deep system is formed in the calf by the union of the posterior tibial and fibular trunks which anastomose with a separate anterior tibial trunk. It collects lymph from the periosteum, fascias, and muscles.

There is a strictly regional pattern of drainage (26). Injection of dye into the web space of hallux shows the medial superficial group which runs into the subcutis of the lateral aspect of the heel the lateral superficial trunk. The deep lymphatics are visualized by injecting dye into the deep structures of the sole of the foot. In the normal individual, no anastomoses can be demonstrated between the subfascial and epifascial lymphatic trunks (26). Thus, lymph sampled from a collecting trunk in the calf is, derived from a defined area and mass of tissues. Most suitable for chronic cannula-

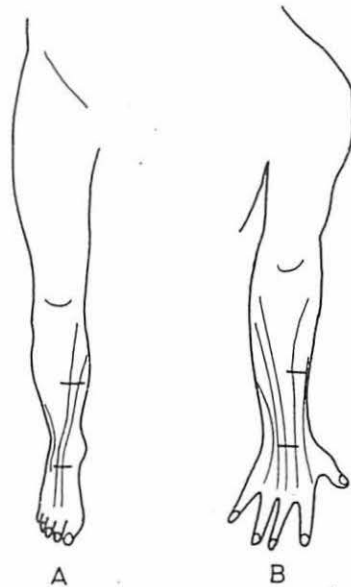


Fig. 1 Diagram of superficial lymphatic system in leg (a) and upper limb (b). Thick transverse lines indicate skin incisions where collecting trunks can be found for chronic cannulation.

tion in the lower limb are the superficial medial group trunks (Fig. 1a).

#### *Anatomy of Lymphatics of the Upper Limb*

The digital lymphatic plexuses are drained by vessels which run along the outer margin of the fingers toward the web. Here they are joined by vessels from the distal palm and pass toward the dorsal surface of the hand. Collecting vessels from the palmar plexus unite to form a trunk which runs around the metacarpal bone of the fifth finger to join the dorsal vessels of the same finger. The ulnar group of lymphatics drains the third, fourth, and fifth fingers as well as the ulnar side of the hand and forearm. These vessels run along the basilic vein to the cubital region. The radial group of lymphatics drains the first and second digits and the radial side of the hand and forearm. Lymphatics running on the dorsal radial part of the forearm are most convenient for chronic cannulation (Fig. 1b).

### Chronic Cannulation of the Lymphatics of the Leg

The method has been worked out by Engeset et al. (5) Olszewski et al. cannulated thigh (22) and foot (25) lymphatics in patients with pathological alterations of the peripheral lymph vessels. Lymphatic cannulation can be performed in connection with lymphography (7); extra surgical intervention for cannulation is then unnecessary. In this way lymph can be collected over a period of days in a large group of patients with various pathologies. Leg lymph can be easily obtained by cannulation of the superficial collecting trunks on the dorsum of the foot, medial group of calf vessels, in the thigh and groin. For chronic cannulation, however, the lower part of the calf seems to be most suitable.

After injection of patent blue violet in the same way as for routine lymphography, the lymph vessel is exposed. Patent blue coloration of the lymph may, however, influence the results of biochemical studies when colorimetric methods are used; lymph cells may also be damaged. It is preferred to use no dye if lymph composition studies are planned. With experience lymphatic trunks can be found easily even without coloration. Venous stasis of 20 to 30 minutes in the limb before cannulation will dilate lymphatics and aid their dissection.

The cannulation is carried out under strictly sterile conditions. Skin is anesthetized with 1% Xylocaine and a 2 to 3 cm long incision made at the front aspect of the calf about 15 cm above the ankle.

The vessel is exposed and the lymphographic cannula is inserted proximally. After injecting the contrast medium, the cannula is left in the vessel until the patient has been X-rayed. It is then removed and a sterile, siliconized polyethylene Clay-Adams P60 tube with a conical tip, filled with heparin saline solution, is advanced distally through the same opening. If it is not possible to insert the tube through the opening used for lymphography, the vessel is ligated and a new opening is made at a more distal point. Contamination of the tube tip with wound content should be avoided in order to prevent formation of clot in the tube after it is introduced into the lymphatic vessel. A fine thread is put around the vessel containing the tube and tied gently so that lumen, is not occluded. The tube is additionally fastened to the surrounding tissues in the wound with 1 to 2 sutures. The wound is closed and the tube fixed to the skin with adhesive tape. The external tip of the tube is inserted into the sterile disposable syringe through its needle outlet. The plunger is displaced distally so that the whole syringe can be gradually filled with lymph (Fig. 2). The use of syringes has the advantage over the other methods because it protects the sterility of lymph and allows accurate measurement of volume in the course of collection. The danger of lymph evaporation is also avoided since the tube almost occludes the needle outlet of the syringe. According to the requirement, heparin solution, culture media, etc. can be placed at the bottom of the syringe. The syringe is fixed to the leg with adhesive tape. Patients are allowed to walk and there is practically no danger that the tube will slip out. Collection of lymph can be carried out continuously for several days. Special care should only be taken to avoid contamination of the wound and tube during the change of syringes. There may be some clotting of lymph in the tube, especially in cases of low lymph flow. The clot usually protrudes from the tube and can easily be

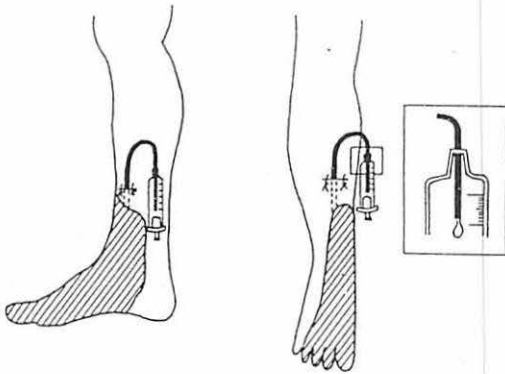


Fig. 2 Leg lymph cannulation. Hatched field indicates area drained by cannulated lymphatic vessel. For lymph collection disposable plastic syringes are used. The P60 Clay-Adams polyethylene tubing fits the opening of any size syringe.

removed by gentle traction with forceps. The temperature of lymph collected in syringes fixed to the calf skin is around 27°C to 31°C. If a low temperature is required, the collecting syringes can be placed in isopore boxes filled with ice and fixed to the leg.

Cannulation of the upper limb lymphatics is practically the same as that described for the lower limb.

#### *Other Methods of Collection of Limb Lymph*

Lymph can also be collected by direct puncture of intact cutaneous lymphatics. The technique has been described by *Threefoot* (33). Briefly, patent blue violet is injected intradermally into the region where lymph is to be collected with a 30-gauge needle. After 10 to 20 minutes, blue streamers are produced. A 23- to 30-gauge needle connected with plastic tubing is then introduced into the dermal lymphatic counter to the current of flow. Use of magnifying glasses facilitates the procedure. The syringe is used for gentle aspiration. Care must, however, be taken not to exert a pressure which would collapse the vessel and draw air into the tubing. The fluid is collected in the tubing and its volume can be directly measured. With this method, only several microliters of lymph can be collected.

In cases with lymphedema with dilated cutaneous lymphatics, lymph samples can be obtained by direct puncture of the skin. Several 23-gauge needles with splint and connected to plastic tubing are inserted into the skin and left for several minutes to allow the bleeding to stop. Splints are gently removed, needles withdrawn 1 to 2 mm and left for 1 to 2 hours. Using this method several microliters of interstitial fluid and lymph can be obtained from each needle, even without aspiration. Contamination of lymph with blood should be avoided. The hemoglobin concentration in each sample should be measured.

#### *Collection of Lymph for Biochemical and Cellular Studies*

Generally, it is possible to collect lymph in sufficient amounts for protein, electrolytes,

acid-base balance, and gasometric studies. The concentration of immunoglobulins, complement, and other immune proteins can also be measured with routine techniques. Since the collection time for lymph is usually several hours and the temperature in the collecting syringes is around 30°C, the possibility of inactivating enzymes and other temperature-unstable substances should be taken into account.

For collection of lymph cells, heparin should be added to the collecting syringes in concentrations not exceeding 5  $\mu$ /ml. To avoid adherence of macrophages to the walls of cannula and syringe, their surfaces should be siliconized. Leg lymph in normal man contains around 90% small lymphocytes and 10% monocytes and macrophages. There is usually a small admixture of erythrocytes. Cell counts vary from several per microliter to several hundred and depend on individual factors as well as, to some extent, on the volumes of lymph produced.

#### *Lymph flow, Hydrostatic and Oncotic Pressure Measurement*

Lymph flow is measured by determining the volume of lymph collected in the calibrated syringe during one unit of time. Since accurate estimation of the weight of tissues from which lymph is derived is difficult, flow rate cannot be present in units of volume-per-time-per-weight of tissue. This makes it difficult to compare the flow data of the subjects investigated. There are significant differences in lymph flow between individuals and from leg to leg in the same individual due to varying topography and caliber of the cannulated vessels and permeability of exchange vessels (8, 15). The mean lymph flow from a vessel of superficial medial group in the calf for 9 legs was  $0.24 \pm 0.056$  ml/hr (SE) during night rest; it increased 15-fold during walking and 30- to 60-fold when the foot was warmed (8, 23).

Lymph hydrostatic pressure can be measured in the tube from which the lymph is collected. A high-sensitivity, low-pressure transducer should be used. Zeroing must be very accurate. Lymph pressure in leg in the horizontal position ranges between 0 to +1 mmHg;

during walking it rises several mmHg. When the tube is located in the lymphatic in the end-to-end position, the "end pressure" is measured. This pressure will rise during walking but does not fall during rest because the valvular mechanism is located distally to the point of measurement. To measure the "lateral pressure", a T-cannula should be inserted into the lymph vessel; this is relatively difficult technically.

Osmotic pressure of lymph can be measured in the membrane osmometer designed by *Johnsen* (14); samples of 5 to 10 microliter are required.

#### *Interstitial Fluid Collection*

Collection of interstitial fluid is indispensable in studies of transcapillary and interstitial transport of high and low molecular substances and water, and their biochemistry. The concept "interstitial fluid" is, however, not fully understood from compositional and functional points of view. Based on recent biochemical and ultrastructural studies of the substance, a heterogeneous system is suggested for the interstitial space. Two phases, one "colloid-rich and water-poor" and the other "colloid-poor and water-rich" exist together. They remain in a functional equilibrium with each other. The colloid-rich phase is more in contact with the structural components of the connective tissue and contains water-insoluble ground substance. The colloid-poor and water-rich phase contains more soluble mucopolysaccharides and seems to be the faster transport route through the interstitium. Depending on the sampling method, different proportions of two phases may be obtained. Generally, three methods of interstitial fluid sampling may be applied: the capsular, the liquid-paraffin cavity and the wick technique. The techniques are described below. With the capsules implanted into the tissue for several days, more colloid-rich material can be accumulated than during the 1 to 2 hour sampling of interstitial fluid with the liquid-paraffin cavity or the wick method when only the colloid-poor and water-rich phase is collected (12). Different methods of collection, therefore, depend on the results of studies of the

concentration of macromolecular substances, as well as the measurements of the equilibration time for labeled proteins between the intravascular and extravascular space. It should also be remembered that interstitial fluid composition, in part, is determined by local metabolism. Different regions may have a different interstitial fluid composition depending on the metabolic need and the type of metabolic discharge from their cells (17).

#### *Capsular Method*

The method has been used primarily for investigations on animals because of its invasiveness; few data from human studies are available. The technique has been described in detail by *Guyton* (10). It has been slightly modified by various investigators with respect to the size and shape of capsules. The author uses polyvinyl plastic around capsules (diameter: 15 mm; thickness 1 mm) with approximately 100 holes 1.5 mm in diameter. Prior to implantation the capsules were sterilized in the autoclave. At the implantation, should be avoided. The capsule is deposited at least 6 to 8 cm from the incision of the skin. Three to four weeks are allowed for healing. After this time the inner surface of the capsule is lined with fibrous tissue; its central part is filled with fluid. The inflammation caused surgery disappears within 2 weeks after the insertion. Fluid can be removed through a needle introduced through the holes. Each capsule can be sampled only once; those capsule with hemorrhagic content should be excluded.

#### *Liquid-Paraffin Method*

The technique has been described by *Häljamae* (11). The skin surface is cleaned and covered with sterile liquid paraffin. A 3 to 5-mm incision is made through the epidermis; the skin and subcutis are dissected bluntly. The incised cavity is kept filled with liquid paraffin to prevent evaporation. Sampling with quartz pipettes is carried out from the cavity; the mouth of the pipette should always be sealed to prevent evaporation of the collected fluid. With this technique, it is possible to obtain 25 to 30 microliters of fluid.

### *Wick Method*

The method was described by *Aukland* and *Fadnes* (1). Two tiny incisions are made in the skin 4 to 5 cm apart. A multifilament nylon wick with a 0.6-mm diameter is sewn into the subcutaneous tissue through the incisions with a round, blunt needle. After allowing about 1 hour in the subcutaneous tissue for equilibration with the interstitial fluid, the wick is removed. The two ends are cut and the middle part quickly transferred to a preweighed test tube with tight cover. The weight of the wick is determined. The wick is then eluted in 2 ml saline over a 24-hour period. The dry weight of the wick is measured after dilution and drying for 2 hours at 100°C. Dry weight is subtracted from wet weight, and the wick fluid volume calculated by using specific gravity for saline to calculate volume from weight. The fluid volume obtained with this method remains within range of 2 to 10 microliters. The hemoglobin concentration should also be calculated in the wick fluid and expressed as a fraction of venous hemoglobin concentration in order to subtract the possible plasma admixture of measured substances caused by injury to the blood vessel. The method has been used extensively in man for studies of transcapillary protein transport (18, 19).

### *"Skin Window" Method*

A modified "skin window" technique can also be used for collection of interstitial fluid for biochemical studies (4). The horny layer of the epidermis is removed via a high-speed grinder with a sterile grinding wheel. About 5 x 5 mm is abraded; if any bleeding is seen, the lesion is not used. A disk of thick filter paper is used to absorb the fluid. This is then eluted and biochemical studies are carried. The material obtained with this method is a mixture of interstitial fluid and plasma; it is an equivalent of inflammatory exudate.

### *Collection of Interstitial Fluid for Cellular Studies*

Cells can be collected from the interstitial fluid by the "skin window" technique (27). These cells, however, are mostly cells actively

migrating from the blood capillaries through the interstitial space toward the abrasion area. Normally, they are not present in the interstitial fluid in those amounts observed under these experimental conditions.

The epithelium is scraped away from an area 3 to 4 mm in diameter. When the papillary layer of the corium is reached, fine bleeding points are seen. The lesion is covered with a sterile, chemically clean, coverglass and then cardboard. The coverglass is attached to the skin with adhesive tape. The tape is applied in such a way that its center is over the lesion. The exudate cells migrate to the undersurface of the coverglass. After 30 minutes the coverglass is removed, rapidly air-dried, and stained.

In a modification of the technique described above, the epidermis is removed with a high-speed grinder (4) and a paper disk is placed on the lesion for cell harvesting.

The wick method does not seem to be suitable for collecting cells because all of the adherent cells attach to the fine filaments and are difficult to remove.

A promising method for collecting cells from the interstitial fluid in the sponge matrix has been described recently by *Roberts* and *Häyry* (28). The method has been designed, primarily, for studies of effector cells in allograft rejection but seems to be applicable to the physiological studies as well. A viscous cellulose sponge is cut into 3x3x3 mm pieces and washed 10 times in distilled water and 70% ethanol. It is then sterilized and placed in the tissue. The infiltrating cell contents of the sponge is recovered simply by compression. The method still needs to be evaluated in certain pathological conditions.

### *Interstitial Fluid Volume Changes and Interstitial Compliance Measurement*

Changes in interstitial fluid volume can most easily be measured using the plethysmographic technique (13). The whole limb or a part is placed in the plethysmographic chamber which records changes in tissue volume following elevation of venous pressure. Venous pressure is increased by inflating the sphygmomanometer cuff placed around the limb proximal

to the plethysmograph. When venous pressure is elevated, the increase in tissue volume consists of an initial rapid phase followed by a longer-lasting slow phase. The rapid phase reflects pooling of blood within the blood vessels; the slow component is due to accumulation of interstitial fluid produced by acceleration of capillary filtration. The latter is used as a measure of change of the interstitial fluid volume. If the changes in interstitial fluid volume are plotted against the interstitial fluid pressure, the interstitial compliance can be calculated from the slope of the curve.

#### *Interstitial Fluid Pressure Measurement*

The measurement of interstitial fluid pressure has been attempted with a variety of techniques, a saline-filled needle (needle technique) was induced into the subcutaneous tissue and connected to the pressure recorder. It was, however, not possible to obtain a steady recording with the open-ended needle and pressures fluctuated widely.

The other techniques were based on measuring the minimum pressure required to force small volumes of normal saline into tissues through a needle. The pressure measured was assumed to be approximately equal to that existing in the tissues before injection. In human subcutaneous tissue, it was found to range between 0 and +8 cm H<sub>2</sub>O (2, 20). Due to recoil of the tissues disrupted by injection, a positive pressure was always recorded. The recording of interstitial fluid pressure was then affected by solid forces within tissues.

It was noted that saline brought into contact with the interstitial space of the skin through a fine needle was drawn in from the needle (2). This raised the possibility that interstitial fluid pressure might be below atmospheric pressure. *Guyton* (9) established that interstitial fluid pressure was subatmospheric by measuring pressures in perforated capsules chronically implanted into various tissues of animals (capsule technique). The "capsular" fluid remained in continuity and dynamic equilibrium with the extracapsular interstitial fluid across the tissue lining of the inner surface of the capsule. Its pressure and composition have been assumed to correspond to

the interstitial fluid. *Guyton's* method made it possible to demonstrate that capsule pressure varied according to *Starling's* hypothesis when capillary hydrostatic or plasma oncotic pressures were varied. It also helped to study the relationship between interstitial fluid pressure (in the capsule) and interstitial fluid volume. Due to its invasiveness, the capsular method could not be clinically applied.

A method has been described recently (31) which was successfully applied in man to measure interstitial fluid pressures (wick technique). The wick is inserted subcutaneously to establish contact with the interstitial space and pressure is transmitted through the catheter-wick assembly to a manometric recording device. The wick and capsule methods have been thought to measure the hydrostatic pressure of the interstitial space. Since, however, there is little free fluid in the interstitial space, the negative pressure might be generated by the osmotic effect of the macromolecules of ground substance — primarily by the osmotic pressure of trapped mucopolysaccharide molecules (29, 30). A significant correlation between wick and capsule pressures has been demonstrated (29). Wick pressures turned out to be only slightly less negative than capsule pressures.

The pressure values obtained using the wick method in the subcutaneous tissue of limbs in man were +3.8 to -3.3 cm H<sub>2</sub>O (mean: -3.4) (29). In other studies pressures measured in the dorsum of the hand were -3.01 ± 0.5 cm H<sub>2</sub>O, in the foot -3.54 ± 1.74 cm H<sub>2</sub>O, and at the level of deep fascia in the forearm -2.41 ± 0.85 cmH<sub>2</sub>O (30). Wick pressures rise when the subcutaneous tissue is warmed. A negative correlation was observed between wick pressure from the forearm and the subject's plasma colloid osmotic pressure (30).

#### *Technique of Wick Pressure Measurement*

The wick is composed of long-fiber cotton batting which has been sterilized in the autoclave. Before sterilization short and weak cotton fibers are removed by combing. The fibers are held by a silk thread and used to pull the sterilized wick into the end of a sterile catheter. The wick is fitted loosely into the cannula

and protruded 5 mm from its end. A polyethylene cannula (internal diameter: 0.7-1.0 mm) is used. The whole system should be filled with saline: special care should be taken to remove air bubbles. The catheter-wick is then connected to a measuring system. A side arm of the measuring system with a cannula attached serves to measure the zero reference pressure.

Skin is anesthetized with xylocaine and a stab incision (1 to 1.5 mm long) made. Bleeding is stopped by gentle pressure for 2 to 3 minutes. A metal cannula with a splint occluding its lumen and with an obtuse tip is then inserted by blunt dissection through the subcutaneous tissue into the vicinity of muscular fascia. Three or four minutes are then allowed for the blood to clot around the cannula. The splint is removed and catheter-wick inserted into the cannula. The cannula is gently withdrawn from the tissues leaving the catheter-wick in the required plane. The catheter can be pulled back a few millimeters to line the wick up with the cannula. Ten to fifteen minutes are allowed for equilibration. The pressures from the wick can then be recorded. If bleeding was present, the measurements may be affected by the presence of a clot. The catheter should then be removed. No reactions have been described after using the wick method to measure interstitial pressure in man. It should be noted that comparing the values obtained by different investigators with the wick method is possible only when the technique and the equipment are the same.

Oncotic pressure of interstitial fluid can be measured in samples of 5 to 10 microliters with the membrane osmometer designed by *Johnsen* (14).

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*W.L. Olszewski, M.D. Dept. for Surgical Research and Transplantation, Medical Research Center, Polish Academy of Sciences, 02-004 Warsaw, 5 Chalubinskięgo*