It is quite difficult to see how any significant lymphatic concentrating mechanism could be possible in subcutaneous tissue. However, other tissues particularly those with high lymphatic protein could possibly have significant protein concentrating mechanisms.

References

1 *Drinker, C.K., M.E.Field:* Lymphatics, lymph and tissue fluid. Williams and Wilkins, Baltimore 1933
2 *Rusznyak, I., M.Foldi, G.Szabo:* Lymphatics and lymph circulation. Oxford: Pergamon, 1967
3 *Guyton, A.C.:* A concept of negative interstitial fluid pressure based on pressures in implanted perforated capsules. Cir. Res. 12 (1963) 399
4 *Gibson, H., K.A.Gaar:* Dynamics of the implanted capsule. Fed. Proc. 29 (1970) 319
5 *Casley-Smith:* Advances in Lymphology 1975
6 *Hall, J.G., B.Morris, G.Woolley:* Intrinsic rhythmic propulsion of lymph in the unanesthetized sheep. J. Physiol. (Lon) 180 (1965) 336
7 *Pappenheimer, J.R., E.M.Renkin, L.M.Borrero:* Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. Am. J. Physiol. 167 (1951) 13
8 *Intaglietta, M.:* Advances in Lymphology 1975
9 *Zweifach, B.W.:* Physiology of terminal lymphatics in the mesentery. Pflugers Archiv. 336 (1972) 565

Aubrey E. Taylor, University of Mississippi Medical Center, Department of Physiology and Biophysics Jackson, Mississippi 39216

Lymphology 8 (1975) 49-56

Functional Anatomy of the Lymphatic Fluids and Pathways

Simon Rodbard, Paula Feldman

City of Hope Medical Center, Department of Cardiology, 1500 East Duarte Road, Duarte, California 91010

Summary

Summary

The present sets of studies indicate that the fibrous capsule which encloses each tissue module divides the interstitial fluids into an intracapsular pool, and an extracapsular pool. Fluid that filters out of the capsules into the extracapsular clefts is the source of the lymph. Because of the limited permeability of the capsular barrier the composition of lymph differs from that of the capillary ultrafiltrate. Lymphatic vessels are means for the drainage of the extracapsular fluids and other materials. This approach differentiates two entirely separate types of edema: an intracapsular dependent pitting edema and an extracapsular generalized non-pitting edema. Three sets of experiments that support the foregoing hypothesis are briefly presented.

Our studies have led to some new perspectives related to the anatomy, physiology and pathology of the lymph and the lymphatic vascular system (1). The implications inherent in our approach provide for re-assessment of the interstitial fluid pool, the anatomical capsules which separate capillary ultrafiltrates from prelymphatic fluids, and related problems.

Current concepts hold that there is only a single interstitial fluid, from which the lymphatic system extracts a minuscule, nearly vanishing volume for return to the blood stream. Thus, osmotic and hydrostatic forces return approximately 99.9 percent of the capillary ultrafiltrate at the downstream end of the blood capillaries. The remaining one part in a thousand of the

extracapillary fluid, together with a small amount of protein is believed to be an excess which somehow can not be taken back into the capillaries, and this fluid is considered to enter the lymphatic system directly. The lymph rootlets (initial capillaries) are held to be almost universally distributed in the interstitial fluid from which they glean this nearly infinitesimal surplus volume and return it via the long path of the lymphatic roots and trunks to the lymphovenous ducts.

A number of difficulties in the generally accepted concept are noted. For example, it is difficult to understand the need for a widespread special vascular system for the drainage of one part per thousand of the total volume of transcapillary ultrafiltrate. In addition, numerous workers have noted differences in forms of clinical edema. If there were only one locus for extracapillary fluid, only one type of edema should be known.

New Concept

Our approach indicates that the interstitial fluid is divided into two discrete pools. This concept is based on a consideration of the role of the tissue capsules (1).

Tissues. Many of the tissues of the body are separated into relatively small modules each of which consists of a cluster of parenchymal (e.g., muscle or epithelial) cells, the blood capillaries that supply these cells, and their extracellular fluids, all enclosed in a compliant relatively impermeable fibrous connective tissue capsule (Fig. 1) (1). This anatomical module is called a *capillaron*. The artery enters the hilum of the capsule to supply the blood capillaries of the capillaron, and the blood is collected in the vein of the capsule. Nerves enter the capillaron along the trabecular pathways. Lymphatic vessels do not enter the capsule.

Fluids. The capillary blood pressure expresses an ultrafiltrate across the vessel wall. Accumulation of this fluid inside the capsule generates a positive intracapsular fluid pressure. This pressure returns almost all of the extravascular fluid back to the blood at the downstream end of the capillary. The remaining minuscule portion of the intracapsular fluid filters across the relatively impermeable fibrous capsule of the capillaron, and thereby enters the clefts between adjacent capsules. This extracapsular fluid is bound by the immensely hygroscopic ground substances in the clefts. Special extracapsular cells in the clefts that may be related to the ground substances include mast cells, fat cells, and other interstitial cells. The extracapsular clefts or

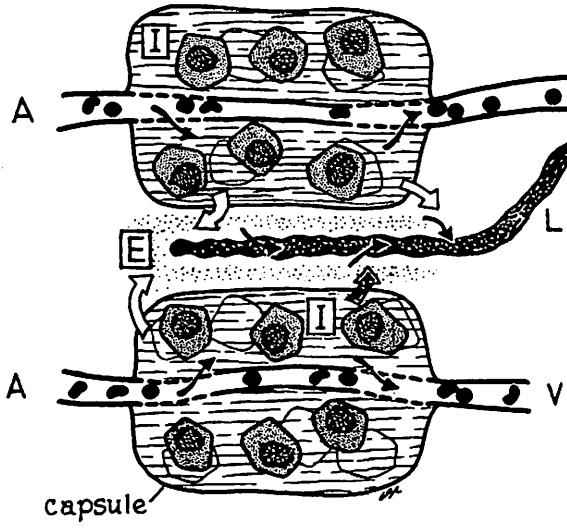

Fig. 1 Two modules demarcated by capsules are shown. Blood is delivered by an arteriole A to a set of capillaries (only one is shown) and flows out of venule V. Ultrafiltrate (black arrow) passes across the permeable capillary wall into the surrounding fluid where it washes the parenchymal cells (nucleated). Most of this fluid returns to the blood at the downstream end of the capillary (black arrow). Some intracapsular fluid (I) ascapes (open arrows) into the extracapsular clefts (E) where it is bound by the ground substance (shaded) and ultimately enters the lymphatic vessels L.

trabeculae are also thoroughfares for passage of arteries, veins, nerves, and other services to more distant capillarons.

The lymphatic vessels are always found in the extracapsular clefts; they never enter inside the capsules (1). The initial lymphatic vessels originate as blindly ending rootlets that consist of a single layer of endothelial cells with a thin basement membrane. Connective tissue fiber attachments hold these rootlets to adjacent capsular membranes. The endothelial cells of the lymphatic capillaries can open in the manner of hinged trap doors, thereby providing for the transport of fluids and particulates out of the extracapsular clefts. The lymphatic vessels are therefore the drainage system of the extracapsular clefts. The fluids percolate through the lymph nodes, enter lymphatic trunks, and ultimately return via lymphovenous ducts to the circulating blood. Particulates and cells tend to be filtered out in the lymph nodes and thereby impeded from entering into the blood stream.

Two Types of Interstitial Fluids. The modular structure of tissues divides the interstitial fluids into two discrete pools: an intracapsular pool, and an extracapsular pool. An excess of accumulated fluid may therefore give rise to one of two entirely separate forms of edema: intracapsular, or extracapsular (1).

Intracapsular edema appears wherever an elevated venous pressure increases the rate of ultrafiltration out of the capillaries and reduces the rate of the return of this fluid to the blood. As a result, fluids accumulate inside the capsules. The volume of such fluid is hydrostatically determined by the increased capillary transmural pressure difference, and by the compliance (volume/pressure) of the capsules. This edema forms at *dependent* sites, as in the legs, or in the dorsum of the supine patient. When venous (intravascular) pressure is lowered, or extravascular (tissue) pressure is raised, as by compressing the tissue, the intracapsular fluid is filtered back across the capillary endothelium directly into the blood. This type of edema "*pits*" as finger pressure is applied.

Extracapsular edema is a more generalized fluid accumulation that is observed after the administration of steroids or when the intrinsic hormonal status produces a high estrogen titre. For example, premenstrual women may gain two or more kilograms. During this phase, the face appears puffy, and the clothes are tight. Similar general weight gains are observed in pregnancy, nephrosis and other conditions. This fluid accumulation appears to be due almost entirely to the extracapsular binding of fluid throughout the body.

In steroid imbalances, increased quantities of ground substances and mucopolysaccharides are produced in the extracapsular clefts. These substances can bind as much as 1000 times their weight in water. Since the water is bound chemically it is not demonstrable in volume dilution studies of the blood stream. The resulting non-pitting, non-dependent edema is distributed in the extracapsular spaces throughout the body and is not available for renal excretion.

A change in steroid balance induces the ground substances to release their water. These fluids can then enter the lymphatic rootlets and return to the blood. Renal filtration then eliminates the excess water from the body.

Local firmly bound fluid accumulations occur in specialized sites such as in the sex skin of certain primates. In these animals, gonodal and related steroids induce a highly localized accumulation of hyaluronates to which fluid is bound to form a firm subcutaneous genital plaque. With the end of estrus the water is released from the hyaluronic acids. Other local extracapsular fluid accumulations include the sudden local sequestrations of water at sites of local trauma, inflammation, and in allergic edemas of the lip, trachea, and vocal cords. These fluids probably accumulate in the extracapsular clefts as pre-lymphatic edemas.

Lymph. The term lymph is sometimes used incorrectly to connote all extravascular fluids. Extravascular fluids that are inside the capsules represent ultrafiltrate that has been modified

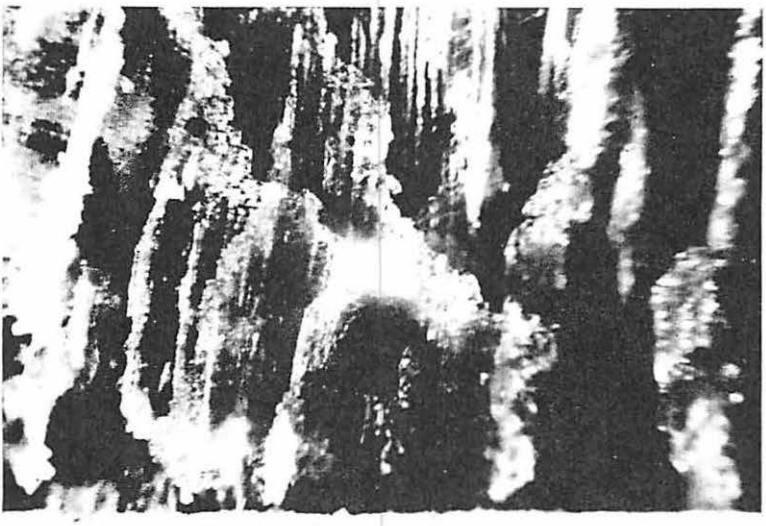

Fig. 2 Cast of skeletal muscle. Incomplete honeycombs are seen in foreground. Negative casts of spaces between the fibrils on the outer margin of the cluster of cells are seen as the long lines.

by the parenchyma; this is best referred to as intracapsular fluid. The fluid held by the ground substances may be properly described as extracapsular or pre-lymphatic fluid. Only those fluids that are in the lymphatic vessels should be referred to as lymph.

Three sets of experiments that offer support for the foregoing concept of tissue structure and for the special extracapsular nature of the lymphatic system are reported. These include (1) the production of casts of the extracapsular clefts and of the lymphatic vessels, (2) the injection of particulates into the tissues and their demonstration in the extracapsular clefts, and (3) the development and utilization of a new reaction that specifically stains the fibrous capsules that separate intracapsular from extracapsular fluids.

1. *Casts of the extracapsular Clefts.* Liquid monomeric methyl methacrylate was mixed with a selected pigment. The addition of catalyst (benzoyl peroxide) and of promotor (dimethyl alanine) initiated polymerization. The mixture was injected by hand through a 22 to 26 gauge needle into various tissues including muscle (2), heart (3), uterus (4), skin and other tissues. Since the viscosity of the monomer is less than that of water, the injected material readily enters the tissues. After the plastic hardens, digestion of the tissues releases a cast of the spaces into which the material was intruded. More than 1000 casts formed in this manner have been studied.

The forms of the casts showed that the injected material had entered only the extracapsular clefts, and that it was excluded from entry into the capsules.

The forms of the casts varied with the tissue injected. In longitudinal skeletal muscle, plastic honeycombs were obtained (Fig. 2). The scalloped edges of the holes in the honeycombs gave evidence that they had contained muscle fibers. This was supported by the fact that longitudinal grooves extended throughout the length of the cast. Scanning electron microscopic photographs of these grooves showed not only the casts of the fibers, but also the negative casts of the blood capillaries that supplied the fibers (Fig. 3).

Casts of small lymphatic vessels originated from the casts of the extracapsular clefts. These small vessel casts joined to form larger casts. Injections of plastic into more complex tissues such as pennate muscles (2), heart (3), uterus (4), and glands generated casts that elucidated some of the more complex structures of these organs, as well as casts of their lymphatic drainage systems. Details of these preparations are presented separately.

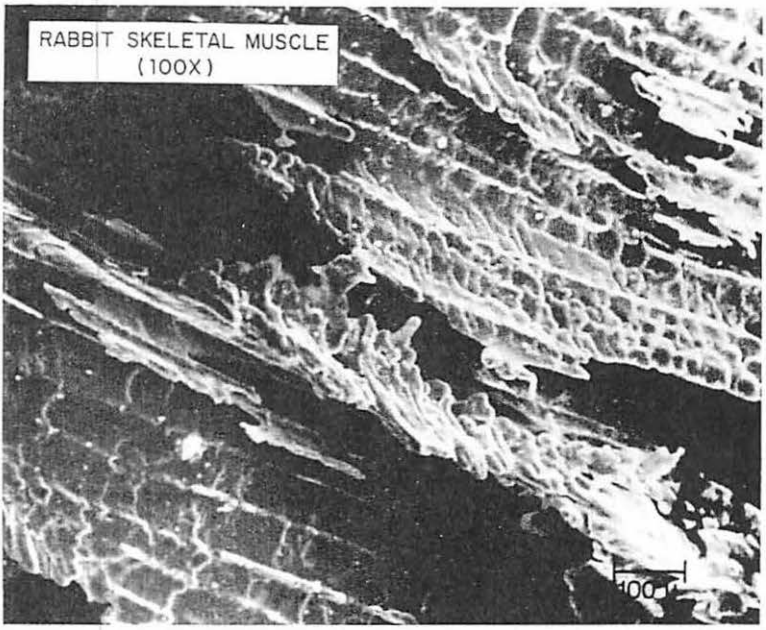

Fig. 3 Scanning electron micrograph of skeletal muscle of Fig. 2. The plastic forms negative casts of muscle fibers by pressing the capsule against the enclosed fibers.

2. *Particulate Injections.* In this series of experiments monomeric liquid plastic into which particulate pigment had been mixed was injected into the tissues. Catalyst and accelerator were not added, with the result that the mixture remained liquid, and much of the liquid plastic leaked out of the needle holes. However, the pigment particles remained in the tissue and thereby marked the sites into which the injectate had been intruded.

The tissues were fixed in formalin, sectioned and stained. The absence of rigid plastic made it possible to obtain adequate sections without the fragmentation that usually occurred in the specimens in which the plastic had hardened.

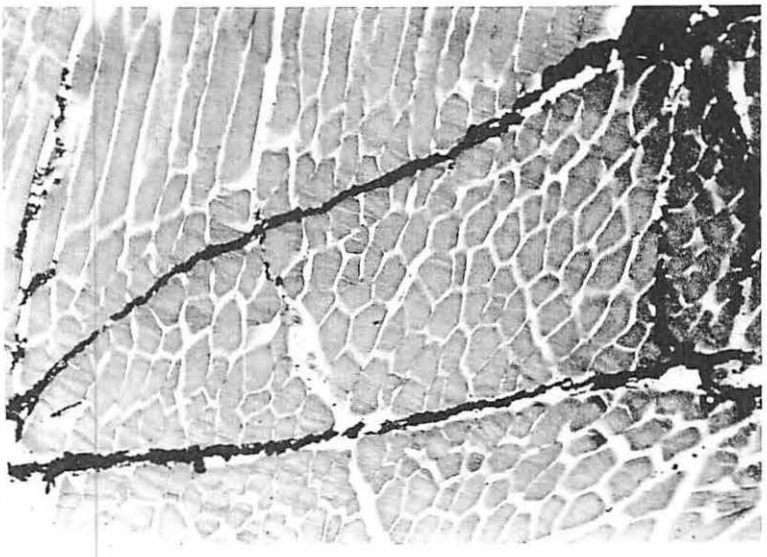

Fig. 4 Pigmented particles injected intraparenchymally are seen as the dark material in the trabecular (extracapsular) clefts. The fibrous capsule prevents the pigment from entering into the intracapsular spaces.

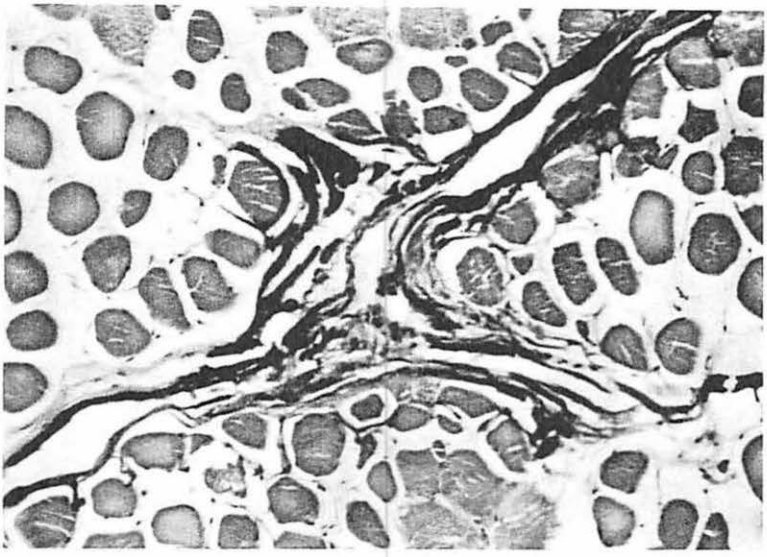

Fig. 5 Deposition of Prussian blue precipitate in the trabecular spaces and lymphatics. No precipitate is seen in the capsules. Discussed in text.

The pigment particles were found in the extracapsular clefts and in the lymphatic vessels (Fig. 4). It was excluded entirely from the inside of the fibrous connective tissue capsules that surrounded each cluster of muscle fibers or glandular cells. This technique also demonstrated that extraordinarily delicate fibrous connective tissue sheets extended between adjacent capsules and prevented the retrograce penetration of the particulates (6). Thus particulates were present in the extracapsular clefts up to the wisps of bridging connective tissue, and none of the particulates were present beyond these bridges.

These findings showed that the material introduced by stab injection entered only the extracapsular clefts. In addition, they demonstrated the presence of delicate sheets of connective tissue that served as barriers to the entry of the injected material retrograde into the trabeculae of the tissue. The delicacy of these fibrous barriers supported the thesis that the injected material entered preformed clefts.

3. *Capsular Staining.* This set of studies was undertaken to evaluate the functional integrity of the fibrous capsules of the capillarons.

Ringer's solution containing one percent potassium ferrocyanide was injected intravenously into anesthetized animals. Ferrocyanide ion, a non-toxic material, was used several decades ago as a means of measuring circulating and extravascular extracellular fluid volume in man. Ferrocyanide passes relatively freely across blood capillary membranes to enter the intracapsular pool of water. It filters more slowly across the capsule in the direction of the extracapsular clefts.

Ringer's solution containing ferric ion was then injected into selected sites. The sites into which the ferric ion was introduced exhibited deep blue streaks, indicating the local precipitation of insoluble ferric ferrocyanide (Prussian blue).

Histological examination of the tissues showed that the outer wall of the capsules had been marked by the precipitate (Fig. 5). Precipitates were also present in the lymphatic vessels of the tissue.

These results show that fibrous capsules separate the intracapsular fluids (ultrafiltrate of the capillaries in which ferrocyanide was present), from the fluids of the extracapsular clefts into which the ferric ion had been introduced. Prussian blue was precipitated at the interface sites

between the intracapsular and extracapsular pools. Some of the particulates also entered adjacent lymphatic rootlets.

Nearly 100 years ago some investigators began to examine the structure of tissues by means of the intraparenchymal injection of gelatin or other masses into tissues. Histological sections indicated that the injected materials had entered special sites (6). *Bartels* (7) vigorously criticized the findings as artefacts, produced *de novo* by the force of injection.

Bartel's criticism inhibited further work on tissue structural analysis based on intraparenchymal injections. The study of the distribution of intraparenchymal injections was thus virtually abandoned even though thousands of intraparenchymal (intradermal, intramuscular) injections are made daily by physicians all over the world. The specific pathways of such intraparenchymal injections have therefore remained unclarified.

Advantages of the present methods over those in use at the turn of the century are that permanent three-dimensional casts of the extracapsular clefts and their lymphatic drainage systems become available for study. The recent development of lymphangiography has demonstrated beyond question that intraparenchymal (intradermal) injections introduce the injected material into the lymphatic system.

Our studies indicate that except for the few capsules impaled on the needles, intraparenchymally injected materials are deposited primarily in the trabecular clefts. The drainage system of these clefts is in the lymphatic system.

Capsular permeability permits water, solutes and some proteins to pass across the capsular barrier. Most students of capillary permeability generally consider that the blood capillary wall is the only barrier between the plasma and the interstitial fluids, and the lymph. They hold that the fluid in the lymphatic vessels is virtually identical with the interstitial fluids. They therefore consider that only the permeability of the blood capillary membrane determines differences in concentrations of ions, proteins in the plasma and in the lymph. The permeability of these capillary membranes is then estimated from the lymph/blood ratio of substances originating in the blood.

The present approach divides the "interstitial" fluids into intracapsular and extracapsular moieties. The membranes of the capsules are considered to be an important semipermeable barrier between the blood capillary ultrafiltrate and the lymph. Since the concentrations of the plasma proteins in the lymph are significantly less than in the plasma, the permeability of the capsule may be limited.

Conclusion

The present sets of studies indicate that the fibrous capsule which encloses each tissue module divides the interstitial fluids into an intracapsular pool, and an extracapsular pool. Fluid that filters out of the capsules into the extracapsular clefts is the source of the lymph. Because of the limited permeability of the capsular barrier the composition of lymph differs from that of the capillary ultrafiltrate. Lymphatic vessels are means for the drainage of the extracapsular fluids and other materials. This approach differentiates two entirely separate types of edema: an intracapsular dependent pitting edema, and an extracapsular generalized non-pitting edema. Three sets of experiments that support the foregoing hypothesis are briefly presented.

References

1 *Rodbard, S.:* The capsular barrier between the interstitial fluid and the source of the lymph. Curr. mod. Biol. 3 (1969) 27-34

2 *Rodbard, S., Taller, S.:* Plastic casts of extracapsular spaces and lymphatic vessels. Med. Exp. 19 (1969) 65-70

3 *Rodbard, S.:* Structure of the ventricular myocardium and conduction system. Amer. J. Cardiol.

32 (1973) 877-879
4 *Rodbard, S., McMahon, N.J., M.Denk:* Uterine structure and lymphatics. Obstet. Gynecol. 38 (1971) 171-179
5 *Rodbard, S.:* Trabecular barriers (frenulae) of the extracapsular clefts. Proc. Soc. Exptl. Biol. & Med. In Press
6 *Rusznyák, I., M.Földi, G.Szabó:* Lymphatics and Lymph Circulation. Pergamon Press, London, 2 nd Edition (1967)
7 *Bartels, P.:* Das Lymphgefäßsystem. Handbuch d. Anatomie d. Menschen. G.Fischer, Jena. In: Lymphatics and Lymph Circulation, Rusznyák, I., Földi,M., and G.Szabó, Pergamon Press, 2nd Edition London (1967) 52-53

Simon Rodbard, M.D., Ph. D., City of Hope Medical Center, Department of Cardiology, 1500 East Duarte Road, Duarte, California 91010

Lymphology 8 (1975) 56-61

Comments on Operations for lower Limb Lymphoedema*

J.B. Kinmonth, J.H. Patrick and A.S. Chilvers

St. Thomas's Hospital Medical School, London, S.E. 1

Summary

Good results have been obtained from surgical operations for lymphoedema of the lower limbs using skin flaps with a blood supply to cover the muscles following reduction of swollen subcutaneous tissue. The Charles operation using free skin grafts for cover is reserved for tropical elephantiasis or patients with local skin in bad condition. A variety of other procedures of physiologic intent have given disappointing results and been abandoned.

The results of 74 operations are reviewed with a view to improving still further the results. The mortality rate was nil and there were only minor complications.

These are comments on some of the operations which we have used for lymphoedema of the lower limbs. Particular attention has been paid to methods of *improving the flap operations.* Technical details, indications and the physiology of the many different operations available for the treatment of lymphoedema have recently been described (*Kinmonth*, 1972) and will not be repeated here.

We have, in recent years, done over a hundred reducing operations using flaps. In these the raw surfaces of muscle and other structures which are left bare after excision of the oedematous spongey subcutaneous tissues are covered by flaps of skin. Enough subcutaneous tissue is retained in the flaps to carry and maintain the blood supply. These skin flap operations are essentially reducing or excisional operations and must be distinguished from pedicle grafting operations of the *Gillies* type which aimed chiefly at improving the physiology. The results considered here are from a group of 74 where the records were easily available for review.

In recent years we have favoured some type of flap operation, particularly variations on the *Thompson* buried dermis flap.

*Based on a communication to the International Society of Lymphology IVth Congress, at Tucson, Arizona, March 1973.