HAS MODERN TECHNOLOGY CHANGED OUR CONCEPT OF LYMPH FORMATION?

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The process of lymph formation is basic to all thinking on lymphatic function, yet it still arouses the curiosity of researchers. Indeed, the mechanisms involved in this seemingly simple phenomenon have aroused the curiosity of a great number of investigators since the discovery of the lymphatic vessels in the early part of the 17th century. From this time our understanding of lymph formation progressed gradually up to the end of World War II, more or less in parallel with the leisurely pace of research. Since 1945, however, the tempo of research has quickened with onset of the so-called technological era. It is pertinent to ask, some 40 years later, whether our concept of lymph formation has changed as a result of advances in technology and an increased tempo of research.

EARLY CONCEPTS 1627-1945

Before trying to answer this question, it will be helpful to reflect on some of the earlier ideas of lymph formation. In his description of the lacteals in 1627 Asellius postulated that chyle from the intestinal lumen entered these vessels by a suction-like action of their open mouths, which he likened to the action of leeches sucking blood, and then passed to the liver by a suction-action of the blood vessels and of the liver itself (1). When the lymphatic vessels, the vasa serosa, were discovered in other tissues in 1653 by Rudbeck in Uppsala (2) and Bartholin in Copenhagen

(3), the view was expressed (by Bartholin) that after transuding through the blood vessel wall the solids of the blood were used by the tissues, a concept in conformity with the teaching of Galen, and the water which remained was returned to the blood by the lymphatic vessels. Thus, even at a time before Harvey's description of the circulation of the blood had been generally accepted, it was thought that lymph was formed as a result of transudation of materials through the walls of the blood vessels. Moreover, the water, which remained as tissue fluid, entered the lymphatic vessels through openings and moved centrally by forces, or a pressure gradient, created by suction. Although the mechanism of this action was ill-understood, the concept is intriguing to modern lymphologists who, in 1986, continue to debate the process of lymph formation.

The ultimate acceptance of Harvey's concept of the circulation of the blood and the discovery of the thoracic duct gave researchers a further basis from which the phenomenon of lymph formation could be considered. It was not, however, until a century or more later that a clearer picture emerged. Although Rudbeck had noticed that lymph had a salty taste and clotted like blood, it was William Hewson in the 1770's who showed that blood, tissue fluid (from the pleural and peritoneal cavities) and lymph (from lymphatic vessels of the

leg) all contained salts and certain mucilaginous substances, later known as proteins (4). These mucilaginous substances were always present in lymph and tissue fluid, but in concentrations which, though varying with circumstances, were always less than those in plasma. In postulating that these three fluids all belonged to the same species of fluid, he maintained that tissue fluid was formed by transudation through the walls of the small blood vessels— "through organized passages which not only transmit the lymph (tissue fluid) from the blood but change its properties and make it assume different appearances in different circumstances of health." Hewson was discussing the lower-than-normal mucilaginous content of lymph in dropsy and the high content in inflammation, observations for which he had no explanation.

Considerable thought was also given to the manner in which tissue fluid entered the terminal lymphatic vessels to become lymph. Although, at this time, it was generally agreed that lymph was derived from blood plasma, there was considerable controversy concerning the mechanism of its formation. One view, which had persisted since the days of Bartholin, was that there were direct connections between arteries and lymphatics. The more generally accepted view, however, was that lymph was formed by the entrance of tissue fluid into the lymphatic vessels by a process of absorption or suction. Hewson's fellow anatomists in London, William Hunter and William Cruickshank, were most ardent proponents of this view, even maintaining that the veins played no part in absorption. The lymphatics became known as the absorbing vessels and the mechanism of their suction-like action was the subject of much discussion. Hewson likened the process to the absorption of chyle through orifices in the lacteals of the intestinal villi and he put forward the interesting hypothesis that during absorption the blood vessels in the villi become turgid by which means the orifices of the lacteals are held open. He thought a similar mechanism might apply to lymphatics in other tissues: "Perhaps such

membranes as the pleura, peritoneum etc. to answer the same purpose may have a network of blood vessels surrounding the absorbing pores, which reticulation, by its turgency, may make the pores stand rigidly open as those we have observed upon the villi." Hewson was aware that the openings in the terminal lymphatics had to be held open by some means, so that the tissue fluid could enter. He then described how tissue fluid entered the lymphatic, "as it would a capillary tube, at least as far as the first pair of valves ... the vessels which (then) convey the fluid onwards are believed to have muscular fibres, which being stimulated by the fluid may contract peristaltically and pass the fluid forwards from one pair of valves to another." Hewson had observed the rhythmic contractility of the larger lymphatic vessels. His experiments and ideas certainly put investigators on the right road to follow in their search for a clearer understanding of lymph formation.

Almost a century later Carl Ludwig introduced the manometer for measuring arterial blood pressure. This measurement influenced his thinking when he put forward his mechanical theory of lymph formation (5) which postulated the filtration of blood plasma through the walls of the blood capillaries. The filtering force, the capillary blood pressure, was thought to continue as a positive pressure into the tissue fluid driving this fluid into the open lymphatic capillaries to form lymph. No capillary or tissue pressures, however, were actually measured. At about the same time, others were focusing attention on the cellular structure of epithelium and of what was to be called endothelium. In 1862 von Recklinghausen described his experiments in which he used silver nitrate to outline the cells that make up the walls of the small lymphatic vessels (6). These findings stimulated considerable discussion of the relationship of the lymphatic capillaries to tissue fluid and the mechanism of entry of tissue fluid into these vessels. It seemed from histological preparations that the lymphatic system was closed to the tissue fluid compartment; yet the concept that tissue

fluid was continuous with lymph through openings persisted until the beginning of the present century (7).

Meanwhile, Ludwig's mechanical theory of lymph formation received a setback later in the 19th century when Rudolf Heidenhain challenged the concept. He put forward an entirely new theory, that of lymph secretion (8). However, Ernest Starling, who worked with Heidenhain in Breslau in 1892, put a different interpretation on the results of Heidenhain's experiments when he repeated them on his return to London. He rejected secretion as a mechanism of lymph formation and reverted to Ludwig's concept. He modified this when, in 1896, while considering how tissue fluid could be absorbed, he made the important suggestion that the osmotic pressure of the plasma proteins, though small, played an essential role in the formation of lymph (9). For the first time actual measurements of filtration pressures were made. Starling got an indirect measure of capillary pressure and he also measured the osmotic pressure of the plasma proteins. As with Ludwig before him, he was mainly interested in the formation of tissue fluid (10) which, once formed, was regarded as free to enter the lymphatic capillaries. Starling accepted Ludwig's view of the entry of tissue fluid into these vessels: "As the blood flows through the capillaries at a given pressure, a certain proportion of its fluid constituents filters through the vessel wall, forming a transudation which is still under a certain amount of pressure, and it is this remaining pressure which causes the onward flow of lymph. Hence the ultimate cause of the lymph flow must be looked for in the energy of the heart's contraction." This concept of lymph formation was not only in opposition to Heidenhain's secretion theory, but also to the earlier suction theory. Moreover, the controversy whether the terminal lymphatics were open or closed to the tissue spaces had not been resolved. The views of Ludwig and of Starling veered towards an open system. However, some investigators felt that, if the vessels proved to be a closed system, the tissue pressure would cause the thin-walled lym-

phatic capillaries to collapse. To combat this criticism, Starling pointed out that anatomists had shown that in certain situations "the walls of the lymphatics are connected by strands of elastic fibres with the surrounding connective tissue so that a rise of tension in the meshes of the latter will only drag the walls of the lymphatics further apart, and thus increase rather than diminish the lumen."

Although Heidenhain's followers continued to support the secretion theory well into the present century, Starling's observations and logical reasoning ensured that attention would be focused on the plasma proteins in any research concerning the mechanism involved in lymph formation. During World War I (1914-1918) the treatment of wound shock with protein solutions supported Starling's concept of fluid exchange across the walls of blood capillaries. After the end of this war further strong support was forthcoming when direct measurements of the pressures involved were made by Landis and his colleagues (11). It was at this time, in the mid-1920's, that a further stimulus was given for experiments on lymph formation. In 1926 Cecil Drinker, while working in Copenhagen with August Krogh, was impressed with the very rapid formation of lymph in the frog and with the fact that the lymph always contained protein. On his return to Harvard he decided to concentrate on the function of the mammalian lymphatics in absorbing fluid and particulate material from the tissue spaces. Drinker devised techniques to collect lymph from many different tissues of the body. In his monograph with Madeleine Field, published in 1933, he concluded that proteins are present in lymph wherever it is collected and that they have their origin in the filtrate from blood capillaries (12), confirming Hewson's findings over 150 years earlier. They wrote, "In our view the lymph capillaries are complete vessels but their content is identical with the tissue fluid outside them ... capillary lymph and tissue fluid are considered to exist in a common reservoir." The tissue fluid pressure was measured and it was thought that a positive

gradient existed between the tissue fluid and lymph. The question was again asked, "in these circumstances, what prevents the collapse of the small thin-walled lymphatic vessels?" This question had intrigued Hewson and he put forward an intriguing hypothesis. With the development of histology, Gaskell showed in 1876 that elastic fibres connected the walls of the lymphatic capillaries and could hold the vessels open (13). In 1935 Pullinger and Florey (14) reinvestigated the structure of the lymphatic capillaries by light microscopy and showed that collagen fibres were attached to the outer walls of these vessels, which were by this time considered to be much more permeable to protein than the blood capillaries. The precise mechanism of permeability, however, was not understood.

In 1941, in his monograph with Joseph Yoffey, Drinker reaffirmed his belief that the experimental evidence supported his view that tissue fluid and lymph are approximately the same in composition (15). In discussing the evolution of the circulation and the steps whereby the lymphatic system has become comparatively isolated from the blood vascular system, Drinker and Yoffey write, "There can be no doubt that these steps have been actuated by physiological necessity and the possibilities are that the main factor in this necessity has been the need for a specialized mechanism to return to the bloodstream blood proteins which have leaked from the blood capillaries." This was the position in 1945 when, with the end of World War II, the technological era, in which we find ourselves today, began. Modern technology has given us more sophisticated tools with which to challenge the concept of lymph formation as enunciated by Drinker and Yoffey. These include: polyethylene tubing for collecting lymph over long periods of time; labelling of proteins with radioactive isotopes and other markers; the separation of plasma proteins into their several components by electrophoresis and of lipoproteins by electrophoresis and ultracentrifugation; electron microscopy which has added a new dimension to structure; more refined methods for the accurate measurement of pressures and the use of computers for the analysis of data.

THE ROLE OF MODERN TECHNOLOGY DURING THE PERIOD 1945-1965

Several questions required further investigation. For example, can the view that tissue fluid enters a lymphatic capillary along a gradient of pressure be justified; do all proteins in the plasma take part in the so-called extravascular circulation and is this strictly a one-way traffic of protein — from plasma to tissue fluid to lymph to plasma; what is the structural nature of the lymphatics which makes these vessels specialized for the return of protein from the tissue fluid; is the composition of lymph the same as that of the tissue fluid from which it is derived? In the 20 years from 1945 many experiments were aimed at answering these questions. In 1965 the results of many of these experiments were discussed at the first international conference on lymph, held in New Orleans and sponsored by Tulane University in honor of that distinguished lymphologist, H.S. Mayerson (16).

The question of the pressure gradient from tissue fluid to lymph was investigated by McMaster. In 1947 he measured the interstitial fluid pressure directly by inserting a fine needle into the skin of a mouse's ear and at the same time measured the pressure inside a small lymphatic by the same technique (17). He found that there was a positive gradient of pressure from the interstitial fluid to lymph, which increased in magnitude when the ear was made acutely edematous. These experiments suggested that, as far as pressures were concerned, the assumptions made by earlier workers were confirmed.

With regard to the question of the one-way traffic of tissue fluid protein to lymph, the problem was approached by studying the absorption of proteins from the serous cavities, which obviated the criticism of injection into a solid tissue. The right lymph duct and the thoracic duct were cannulated in the cat and it was found that in these preparations very little homologous plasma

protein, introduced into the pleural cavity, entered the bloodstream (18). A little later, labelled homologous plasma proteins were introduced into the peritoneal cavity in a similar preparation (19). The results of these experiments were again striking; with the collection of lymph from these two main lymph channels, very little labelled protein entered the bloodstream. What little did find its way into the bloodstream was thought to enter by way of some small branches of the right lymph duct or the thoracic duct which had not been ligated when the main channels were cannulated. To show that this might be so the experiments were repeated in the rat in which the lymphatic vessels on right and left sides were ligated and the veins then stripped where these vessels enter at the base of the neck (20). In such preparations the amount of labelled protein entering the bloodstream was either unmeasurable or very small. These experiments impressed on me the predominance of the lymphatic vessels in the absorption of protein from the extravascular fluid, and seemed to confirm Drinker's concept of the role of the lymphatic vessels. We could not state categorically, however, that no protein re-entered the blood vessels directly. Other experiments, in which the proteins were separated by improved techniques of electrophoresis and the lipoproteins by electrophoresis or ultracentrifugation, showed that all the proteins and lipoproteins detectable in plasma were also present in lymph from various regions of the body, and presumably took part in the extravascular circulation from plasma to tissue fluid and back to the plasma by the lymphatic vessels (21).

The use of the electron microscope to study the fine structure of biological tissues seemed to resolve the problem of the so-called "specialized" function of the lymphatic capillaries in absorbing protein. In 1961 Casley-Smith and Florey showed that the endothelial junctions of the lymphatic capillaries of the diaphragm were often open or were capable of being opened when the amount of tissue fluid increased (22). In this way the lymphatic capillaries were specialized and provided a preferential channel for the absorption of macromolecules, as well as particulate material, from the tissue fluid. It is also interesting to note that in edema, when increased absorption is required, the lymphatic capillary junctions are held open by special fibril attachments, supporting the earlier concepts which were based on observations with the light microscope (23).

THE ERA OF THE INTERNATIONAL SOCIETY OF LYMPHOLOGY 1966-1986

It seemed, therefore, at the time of the New Orleans Conference in 1965, that many of the queries that had been raised concerning Drinker's concept of the role of the lymphatics, had been studied and much progress made towards their solution. So successful was this conference that in 1966 the International Society of Lymphology was established. It was hoped that its biennial congresses would bring together lymphologists from a wide variety of disciplines for discussion of the many problems that required further investigation. In the ensuing 20 years, to 1986, ten such congresses have been held in many parts of the world and have been successful in stimulating debate and interest in several fields of lymphology.

Interstitial fluid pressure: Of the many aspects of lymphatic function studied since the New Orleans Conference, a great deal of interest has been focused on the interstitial fluid — its nature, its composition and the pressure it exerts. It had long been known that interstitial fluid exists in two forms, "free" fluid and "captured" fluid, as McMaster and Parsons described it in 1939 (24). The pressure that this fluid was thought to exert had been measured in various tissues by the well-known needle technique. In subcutaneous tissue with the subject at rest the reading was usually one or two centimeters of water above atmospheric pressure. In 1963, however, Guyton introduced a new technique, the chronically implanted capsule, to measure the subcutaneous interstitial fluid pressure in the dog; he found that the reading was

normally negative, on average — 6.4mmHg (25). This finding has been confirmed not only by Guyton and his coworkers but also by several other groups in the dog, rat and rabbit. Another technique, using an implanted wick, introduced by Scholander and his coworkers in 1968, has also given subatmospheric values in normal subcutaneous tissues, though somewhat less negative than the capsule (26).

Which method comes nearest to measuring the true interstitial fluid pressure has been the subject of considerable debate during the past two decades. All methods involve an element of trauma, the effect of which is difficult to assess. Wiederhielm (27) has defended the needle technique although he concedes that an element of trauma and the introduction of a small amount of free fluid, especially in some of the earlier measurements, would give readings that were too high. Later measurements in which no fluid was injected and in which micropipettes were used, have given somewhat lower values, but not as low as those obtained by the capsule. Wiederhielm has challenged the results obtained by the capsule method, claiming that in the necessary healing process, mucopolysaccharides of the resulting granulation tissue, by their osmotic action, affect the measurements obtained by this method. Guyton and his colleagues (28,29) explain the negative pressure results by postulating that the total tissue pressure is the sum of the interstitial fluid pressure and the pressure exerted by the solids of a tissue. Whereas the capsule gives a true measure of the pressure of the interstitial fluid, the needle technique measures the total tissue pressure.

As far as we are concerned here, the question which arises is, "How do these findings affect our concept of lymph formation?" In this regard, it should be recognized that a very small accumulation of free fluid causes the interstitial fluid pressure to reach supra-atmospheric levels. It has also been found that in several other tissues, especially those which are enclosed by a tight membrane, the interstitial fluid pressure is normally above atmospheric. In normal subcutaneous tissue, however, it could be argued that if the interstitial fluid pressure is subatmospheric while the pressure in the small lymphatic vessels is above atmospheric, the entrance of tissue fluid into the terminal lymphatics could not be explained by a simple pressure gradient. If free interstitial fluid enters the terminal lymphatics under these circumstances, some other mechanism must be invoked.

Modern suction theories of lymph formation: Two hypotheses have been put forward, each of which is dependent on a suction-like action of the terminal lymphatics. In turn, this action is dependent on the structure of these vessels and on the relation of the macromolecules to this structure. The first hypothesis, elaborated by Casley-Smith over several years (30), envisages an osmotic force sucking tissue fluid into the terminal lymphatics. This osmotic force results from his finding, by electron microscope, that the protein concentration of lymph in the terminal lymphatics is about three times greater than that of the interstitial fluid. Tissue fluid, therefore, enters the terminal lymphatics because of the osmotic effect of the lymph proteins. Also important is the structure of the lymphatic capillaries which ensures that the concentration of protein in the lymph remains greater than that of the tissue fluid. On contraction of these vessels, the junctions close preventing the escape of the large protein molecules but allowing a considerable amount of non-protein fluid to return to the tissue fluid compartment.

The key factor in this hypothesis is the relative concentration of protein in tissue fluid and lymph. It is difficult to get accurate chemical measurements of the composition of these fluids on either side of the wall of a lymphatic capillary in a normal tissue. Nevertheless, some evidence exists which is not in agreement with Casley-Smith's findings. For example, calculations based on several measured parameters, indicate that the protein concentration in the extravascular fluid in the tissues of the body excluding the thoracic and abdominal viscera is of the order of 2.1 g/100ml which is about the concentration found in lymph from these tissues (31); direct measurement of the protein concentrations in very small amounts of interstitial fluid and of lymph

from small lymphatics, collected by micropuncture from the subcutaneous tissue of the hind-leg of the rabbit, showed that these two fluids were identical in compositions (32); the colloid osmotic pressure of tissue fluid and lymph were shown to be the same (33), and in the lung experimental evidence supported the view that initial lymph has the same protein concentration as perimicrovascular free interstitial fluid (34). Another factor in the hypothesis which needs further clarification is that, except in one species of bat, lymphatic capillaries have not been observed to undergo rhythmic contractility.

The other hypothesis, put forward by Guyton and his colleagues (35), also envisages a suction-like action of the terminal lymphatics. They have shown that at interstitial fluid pressures of -6 to -7mmHg there is very little lymph flow, but as this pressure increases to atmospheric pressure, the lymph flow increases rapidly. An important factor in this hypothesis, as with the previous hypothesis, is the structure of the terminal lymphatic vessels. These authors maintain that "the walls of the lymphatic capillaries contain contractile proteins, and the capillaries contract periodically. After contraction is over, the anchoring ligaments pull the capillaries open again creating a suction cycle." It is the recoil of these anchoring fibrils which creates the suction necessary to achieve a negative interstitial fluid pressure.

As with Casley-Smith's hypothesis, a key assumption is the contraction and relaxation of the lymphatic capillaries, a phenomenon that has not been observed except in one species of bat. In putting forward a similar hypothesis, Reddy and his coworkers recognized this difficulty and proposed that the first lymphangion, next to the lymphatic capillary, is the important structural unit in the creation of the suction action (36). This hypothesis is very similar to that suggested by Hewson, more than 200 years earlier. Hewson realized that lymph had to pass the first pair of valves before it could be pumped centrally by what have become known as lymphangions.

Casley-Smith was trying to explain how lymph could be formed in circumstances in which the interstitial fluid pressure was negative and the pressure in the terminal lymphatic positive. No one (except Hogan, as we shall see) has yet shown lymph to be formed in these circumstances. Guyton and his colleagues did not measure the pressure in the lymphatic capillaries when they measured lymph flow at negative interstitial fluid pressures. In experiments in which the interstitial fluid pressure and the intralymphatic capillary pressure were measured simultaneously, they were shown to be approximately the same. For example, in the cat mesentery, pressures in the tissue fluid and in the small lymphatics were about the same, either slightly positive or slightly negative (37). In the bat-wing preparation, Wiederhielm and Weston found an average pressure of +1.3 cm water in tissue fluid and +1.2 in the lymphatic lumen (38), but these results have been criticized on the grounds that free fluid had to be introduced into the tissue before the lymphatic vessels could be observed. More recently, Hogan and Nicoll (39) and Hogan (40), using improved measuring techniques but the same bat-wing preparation in which a minute amount of fluid had to be introduced to visualize and puncture one of the lymphatic bulbs, have again measured simultaneously throughout the contractile cycle the intralymphatic pressure and the interstitial pressure just outside the wall of the bulb. The intralymphatic pressure, as might be expected, was shown to be phasic with the contraction and relaxation components of the contractile cycle. Although the average intralymphatic pressure was +0.39 cm water compared with +0.03 cm water for the interstitial pressure, the results showed that in all 16 experiments the interstitial pressure was greater than the intralymphatic pressure for at least part, on average 43 percent, of the contractile cycle. These results indicated that, irrespective of the average pressures, free interstitial fluid could flow into the lymphatic bulb along a pressure gradient for part of the contractile cycle. On contraction of the bulb, the intralymphatic pressure rose above the in-

terstitial pressure, but the presumed closure of the flap-like junctions and the opening of the lymphatic valves would ensure the flow of lymph centrally. Another important result of Hogan's experiments in which he measured the tissue pressure at some distance from, as well as close to, the wall of a lymphatic capillary was that the formation of lymph during the contractile cycle had a suction action on the interstitial fluid some distance away. In his view this mechanism ensures that, under normal conditions, free interstitial fluid is removed by the lymphatic vessels. He writes, "The initial lymphatic bulbs are fluid sinks, lying at the bottom of a shallow, self-created pressure well, draining fluid in from a distance of at least several hundred micrometers. It is possible that the lymphatics continue to pump fluid away from the tissues against a pressure gradient, until the tissue recoil is just balanced by a sufficiently negative IFP."

Hogan has given experimental evidence which supports the suction hypothesis of Guyton and his coworkers. The crucial factor in Hogan's experiments is the contraction and relaxation of the terminal lymphatic capillary bulbs of the species of bat that he used. When they contract, the fibrils attached to the endothelial cells are presumed to be stretched and on recoil, pull the walls back again creating a suction for tissue fluid to enter along a positive pressure gradient, irrespective of whether the actual interstitial fluid pressure is positive or negative, or whether this pressure, averaged over a contractile cycle, is greater or less than the average pressure in the terminal lymphatic. The concept, therefore, that free interstitial fluid flows from the tissue spaces into the terminal lymphatic capillaries along a pressure gradient has gained the support of direct experimental evidence in the bat-wing preparation. The question to be resolved is whether this process functions in other mammals in which the terminal lymphatic capillaries are not contractile. It is possible that the rhythmic contractility of the first lymphangion, as suggested by some researchers, could bring about the same suction effect. In those experiments in which the pressures have been measured, the tissues have been at complete rest. It is well known that muscular contraction increases tissue tension and is an important factor in promoting lymph propulsion. Extrinsic mechanical factors may therefore have an effect on non-contractile lymphatic capillaries similar to that of the intrinsic contractility of the lymphatic bulbs of the bat-wing. While further experimental evidence is required, it seems that modern technology is bringing us closer to an understanding of the forces by which the lymphatic vessels, under normal circumstances, prevent the accumulation of free interstitial fluid.

Other possible mechanisms of protein absorption: Whereas the ultrastructure of the intercellular junctions of the lymphatic capillaries has been shown to be an essential element in the suction hypothesis, some morphologists feel that other structural properties of the endothelial cells may also be implicated in the return of tissue fluid protein to the bloodstream. For example, O'Morchoe and his colleagues have suggested, on morphological evidence, that the pathway of lymph formation in the kidney and liver may be mainly the intracytoplasmic vesicles of the lymphatic endothelium and only to a lesser extent the intercellular junctions (41-43). Ever since Palade demonstrated the cytoplasmic vesicles in the endothelial cells of blood capillaries in 1953 (44), there has been considerable debate concerning the role that these vesicles might play in the transport of protein across both the blood and lymphatic capillaries. The concept that tissue fluid enters lymphatics mainly through the open or loosely-bonded junctions has received fairly general acceptance, although the vesicular route could not be rejected completely. The suggestion now being made is that in the liver and kidney, where the concentration of tissue fluid protein is high, the lymphatic endothelial cells contain a relatively large number of vesicles and relatively few open junctions. Whether this finding is associated with a relatively large

transfer of tissue fluid protein to lymph by the vesicular route is, however, yet to be proved. The proponents of this hypothesis, nevertheless, support the view that the suction mechanism, which involves the intercellular junctions, plays an essential role in ensuring the maintenance of the fluid balance in these tissues.

Whatever the mechanism for entry of tissue fluid protein into the lymphatic capillaries might eventually prove to be, there is at present fairly general acceptance of the concept that one of the functions of these vessels is the return of free interstitial fluid and protein to the bloodstream. Not so generally accepted, however, is the concept that the lymphatic vessels are the only means by which tissue fluid protein is so returned. Some morphologists have postulated, on electron microscopic evidence in relation to injected markers, that in tissues with a high proportion of fenestrated blood capillaries, proteins may pass from the bloodstream into the tissue fluid and also in the reverse direction through the fenestrae. For example, Casley-Smith (30), reviewing the role of the fenestrae, has supported the hypothesis whereby "the arterial limb fenestrae allow much fluid and macromolecules to enter the tissues, but that by far the greater proportions of these are taken up via the fenestrae on the venous limbs, rather than passing to the lymphatics. Thus there would be a very large local circulation of both fluid and protein." He does concede, however, that in these tissues, which include the intestinal tract, kidney and liver, lymphatics are necessary to remove "the excess of this protein, an amount which is quantitatively small but qualitatively vital."

It would be interesting to know what mechanisms control the amount of protein reabsorbed through the fenestrae and that by the lymphatics. In the villi of the small intestine, for example, a network of blood capillaries, both arterial and venous limbs, surrounds the lacteal, so that any protein that leaves the blood capillaries through fenestrae would come into close association with both venous blood capillaries and the lacteals. Equally, any protein absorbed un-

changed from the intestine would also come in contact with both types of vessel. Whether the proponents of the fenestrae theory believe that the rates of absorption of proteins into the blood and into the lymph depend simply on the different rates of blood and lymph flow is not clear. If this were so, it would be difficult to explain why in new-born ruminant colostrum ∂-globulins, which are absorbed intact through the mucosa of the villi, are then taken up by the lacteals rather than by the blood capillaries (45,46).

Physiological experiments have also been devised to ascertain whether the direct venous route plays a role in the return of tissue fluid protein to the bloodstream. Injections of labelled proteins into a tissue have been avoided in order to exclude any criticism of probable direct injection into ruptured blood vessels. Instead, the fate of enzymes released naturally from tissue cells in various tissues has been determined. My colleagues and I found that the enzyme, lactic acid dehydrogenase, released into the tissue fluid after a period of ischemia of a limb, was taken up only by the lymphatic vessels (47), but in the liver acid hydrolases, released in profound hemorrhagic shock, entered the bloodstream even when the hepatic and thoracic lymph ducts were cannulated (48). Szabo and his colleagues have shown that enzymes released during muscular contraction of a limb or following ischemia of the kidney, are taken up not only by the lymphatic vessels but also by the direct venous route (49,50). In the kidney the ratio of venous to lymphatic absorption was much greater than in the leg. Evidence has also shown that labelled ∂-globulin, having entered the tissue fluid of skeletal muscle, could be returned to the bloodstream by the direct venous route (51). Although these experiments suggest that tissue fluid protein might be returned to the bloodstream directly, more so in some tissues than in others, the mechanisms involved have not been elucidated. The relatively greater return by the venous route in the liver and kidney points to the fenestrae being involved, but such a suggestion is purely hypothetical at present.

Lymphatics vs veins in protein absorption: It is interesting to note how, over the centuries, advances in science and technology have influenced ideas concerning the relative roles of lymphatics and veins in absorption. Harvey, in the 17th century, rejected the need for a special system of absorbing vessels since he thought that veins were capable of performing this function. In the 18th century the anatomists postulated that the lymphatics formed the sole absorbing system and that the veins played no role in absorption either from the gut or from other tissues. With the discovery of the proteins in the early part of the 19th century and of their importance in the maintenance of fluid balance across the capillary wall later in that century, the concept was established by the middle of the present century that the lymphatic vessels were solely responsible for returning tissue fluid protein to the bloodstream. Now in the second half of the 20th century the morphologists, with some support from the physiologists, are postulating that in certain tissues the greater part of the protein in the interstitial fluid is transported to the bloodstream by the venous route, but that the lymphatic vessels are nevertheless essential in maintaining the fluid balance in those tissues. No doubt the proteins in relation to lymphatic structure will continue to attract the attention of many investigators interested in the mechanism of lymph formation. Although the mode of uptake of protein from the tissue fluid remains controversial, researchers have less difficulty in accepting the view that larger complexes such as chylomicrons, particles such as bacteria, or cells such as erythrocytes and lymphocytes are taken up from the free interstitial fluid by the lymphatic capillaries, and that entry into these vessels is by way of the intercellular junctions.

Of the many modern advances in technology used in the study of lymph formation, the advent of the electron microscope must rank high. Just as the introduction of the light microscope in the 17th century added a new dimension to our understanding of structure and of the relationship of function to structure, so in the 20th century the electron microscope has added a further dimension to this understanding. Whereas Malpighi, in the very early days of the light microscope, employed this instrument to observe living phenomena, the electron microscope has been used mainly with highly processed, non-living tissues. Movement of proteins in the living state has been deduced from the position of various foreign markers in these processed tissues. In reviewing the difficulties in interpreting such observations, Bundgaard writes "The study of concentration profiles of electron-dense tracers is therefore a hazardous undertaking, and a critical attitude toward such information is important" (52). It is also possible that, even if the electron-dense markers are approximately the same size as the proteins, they may behave differently in their movement throughout the extracellular fluid. I was reading recently a lecture by Dr. James Blundell, given at Guy's Hospital in 1827 (53). He was lecturing on the value of blood transfusion in midwifery. This form of therapy for certain conditions was sometimes very successful and sometimes fraught with disastrous consequences. Blundell did not know that the reason for this unpredictable behavior rested with different receptors on the surface of seemingly structurally-identical red blood cells. However, he realized that the problem that baffled him would eventually be resolved by further research. He finished his lectures thus, "The more discussion, the more objection and defence the operation (blood transfusion) has to undergo, the better. If it be grounded in error, let it perish; if in just principles, it must survive. From the most violent conflicts of opinion, truth has nothing to fear; though long to us, to her a thousand years are but as one day — a point — a nothing in the eternity of her duration."

For almost 400 years our understanding of the mechanism of lymph formation has progressed with advances, not only in anatomy and physiology but in all of the natural sciences. So it will be in the future. As some questions seem to be resolved, others appear which need further technical

and scientific discoveries for their resolution. As a multidisciplinary society, the International Society of Lymphology can look forward to exciting congresses far into the future.

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