Lymphology 34 (2001) 51-68

HIGH POINTS IN THE HISTORY OF LYMPHOLOGY 1602-2001*

J.W. Shields

Santa Barbara, California USA

The science and practice of lymphology is sub-divided into four cooperative components: lymph, lymphatics, organized lymphoid tissues and lymphocytes. Each lymphologist is involved in one or more of these parts which evolve sequentially during embryogenesis (1). Therefore, it should prove worthwhile to review briefly the history of each component as it relates to a vital fluid, namely lymph, integral to the circulation, regulation of cell and tissue growth, and immunity in all animal species.

Chronology

In 1602 , Hieronymous Fabricius of Aquapendente *(Fig. 1, left)*, founder of the Science of Embryology and Comparative Anatomy (2), discovered the cloacal bursa in Leghorn chickens, while working on a benevolent project sponsored by his patron, the Archduke of Florence, for improving egg production in Livorno (3). He demonstrated the novel bursa to William Harvey *(Fig. 1, right)*, his student from England, while preparing his drawings and manuscript on the valves in veins which he discovered in 1578 (3-6).

In 1616, Harvey proceeded to demonstrate that the valves in veins and heart valves prevent the backflow of blood, such that blood circulates continuously from arteries to veins, instead of tidally as advocated by Galen (3). Harvey's demonstration was presented first at the Lumlean Lecture in 1616. Later, adding pointers to Fabrici's drawings of the valves, Harvey published *De Motu Cordis* in1628 (7). This book revolutionized the Science and ultimately the Practice of Medicine (5). In 1651 Harvey in *De Generatione Animalium* proceeded to demonstrate that the embryological development of blood precedes development of the liver in all vertebrates (8). He concluded that the liver was not the prime source of circulating red blood, as was believed previously.

In 1622 in Milan, Gaspar Aselli (Asellius) *(Fig. 2)* discovered intestinal lymphatics in dogs and found that these became filled with white fluid during alimentation (9) *(Fig. 3)*. He postulated that the primary function of these vessels was to carry milk-like fluid into the body after each meal. He likened the process to the mammary secretion of milk, most notably in the artistic colored frontispiece in *De lactibus sine lacteis venis, quarto vasorum mesaraicorum genere, novo invento* (9). This text was published in 1627 after his death through the generosity of Nicholas Peiresc of Aix-en-Provence, who, in turn, confirmed Asellius' findings in humans (10). The late Edigio Tosatti, Professor of Surgery at Genoa, was extremely proud to have had a copy of the original edition of this treatise.

*This article is dedicated to Arnfinn Engeset from Norway and Nils Söderström and Bo Norberg, both from Sweden.

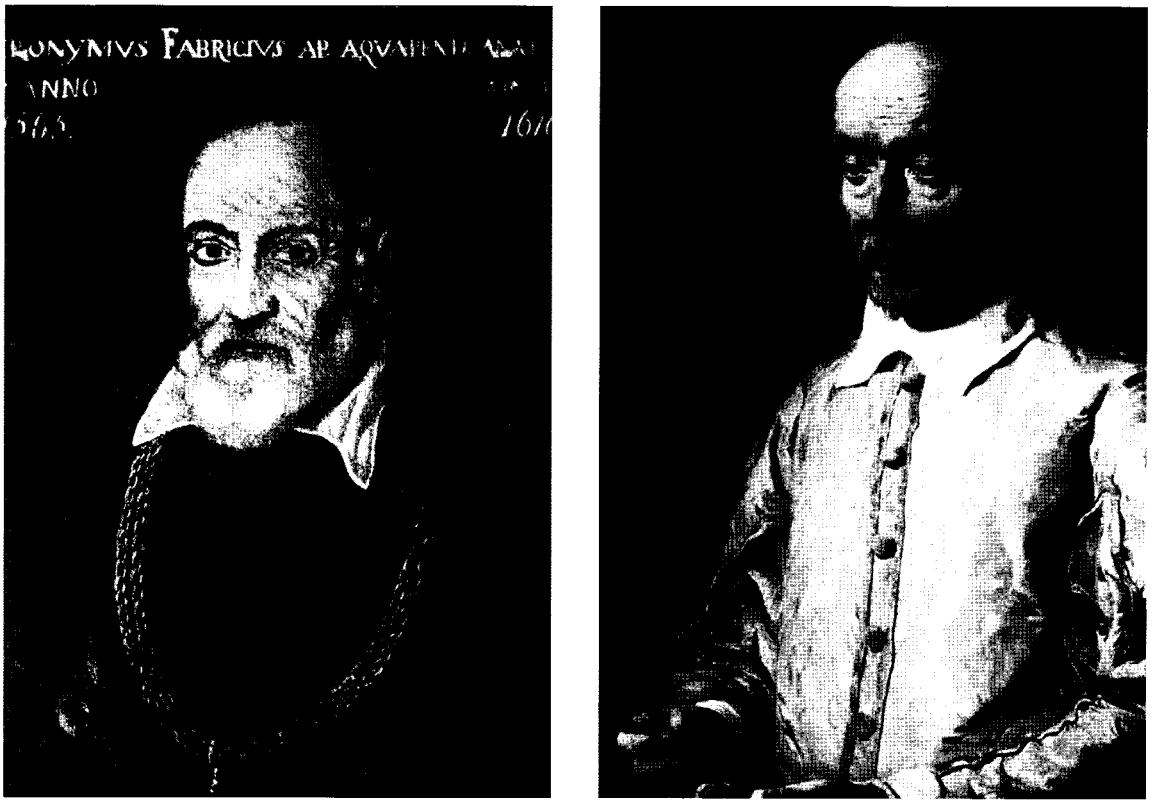

Fig. 1. Left, Fabricius—discoverer of venous valves; right, William Harvey, discoverer of the blood circulation.

Between 1661-1663, Jean Pecquet in Montpellier, Olaus Rudbeck in Upsala *(Fig. 4)*, and Thomas Bartholin in Copenhagen independently discovered that intestinal lymph and lymph from body parts below the diaphragm in mammals drains via the thoracic duct into the left subclavian vein to circulate through the bloodstream (10,11).

In 1666, Marcello Malpighi of Bologna, studying frog lungs, was among the first to use a microscope in medicine. In his book, *Anatomical Treatise on the Structure of the Viscera* (12), he described how arteries connect via capillaries to veins. Also, he was the first to describe red blood cells circulating in lungs, long before hemoglobin was characterized.

In 1778, William Hewson of London, sometimes called the "Father of Hematology" (13), in collaboration with William Cruikshank, John and William Hunter (10) after dissections of many different species of animals during successive stages of development concluded:

1. Lymphatics, not veins, are the primary means of absorption of cell products throughout the body in all animal species (10,13,14).

2. The thymus and lymph glands develop to produce lymph rich in globular particles essential to "normal growth of the body and repair of the constitution" (13-15). Hewson further emphasized such thymic formation of globular particles *(now known as small lymphocytes and globulins)* in his *Experimental Inquiries into the Properties of Blood*, the third part of which was published in 1782 through the generosity of his colleagues five years after Hewson died (13).

Fig. 2. The front piece of the dissertation by Gaspar Aselli of Pavia, discoverer of the intestinal lacteals, published posthumously in 1627.

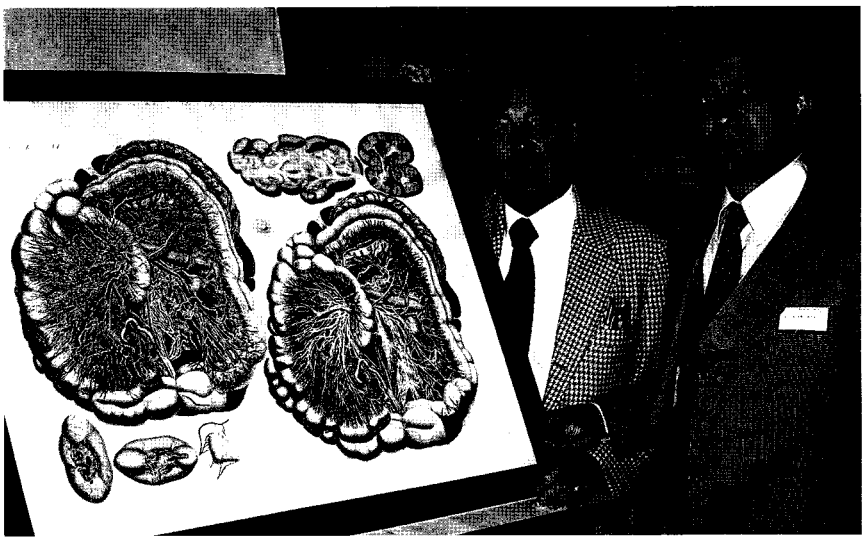

Fig. 3. The author (left) with surgeon Erminio Cariati at Florence, Italy, in 1979 at the 7th Congress of Lymphology besides drawings of Paolo Mascagni.

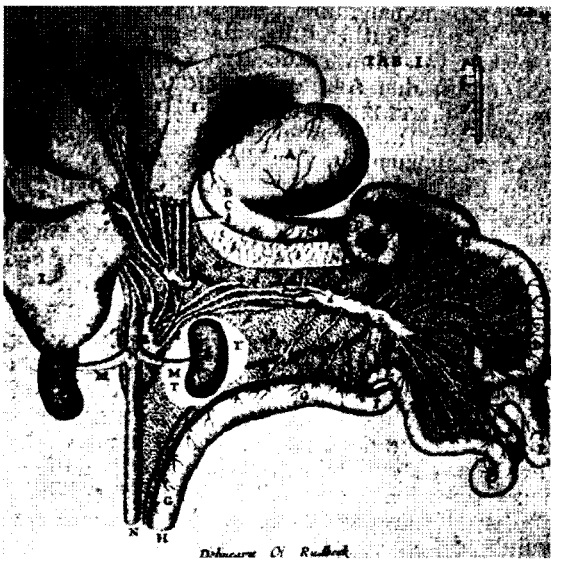

Fig. 4. Olaus Rudbeck of Sweden with his drawings of the lacteals of the mesentery. Along with Jean Pecquet, Thomas Bartholin (who coined the term "lymphatic"), William Hewson, William and John Hunter, and William Cruikshank are usually credited with pioneering observations regarding the structure and function of the lymphatic system during the 17th and 18th Centuries.

In 1787, Paolo Mascagni of Siena meticulously dissected and produced magnificent colored drawings of all major ramifications of the lymphatic system displayed in his *Vasorum Lymphaticorum; Historia et Ichonographia (Fig. 5)*. He was one of the first to recognize that lymphedema may be caused by obstruction within lymph nodes or by incompetent valves.

In 1858, Karl Ludwig of Leipzig *(Fig. 6, left)*, after finding ways to collect lymph from lymphatics other than the thoracic duct and using Berlin Blue as an indicator, concluded that lymph is formed primarily by filtration from blood capillaries (10).

In 1891, Rudolph Heidenhain of Breslau, after comparing lymph constituents with those in circulating blood, concluded that lymph is also actively generated from cells and fibers in tissues (10). [In retrospect, Ludwig's and Heidenhain's contrasting views are probably both valid, because the oxidation of food within cells and tissues creates water, as well as carbon dioxide, quanta of energy and other synthetic byproducts. At the same time, the cells growing between blood capillaries and initial lymphatics require great quantities of dissolved oxygen and soluble nutritive molecules diffusing from blood capillaries in order to respire, produce definitive products, and excrete synthetic products for the benefit of other cells in the body (1,15)].

In 1879, Claude Bernard, author of *Leçons sur les phénomènes de la vie communs aux animaux et vegetaux*, (16) and founder of modern concepts of homeostasis and the milieu intérieur (5,15,17), concluded that all body cells contribute to the formation of lymph and that lymph constitutes the plasma in circulating blood as well as the common means of nutrient exchange sustaining homeostasis throughout the milieu intérieur of all animals (5,15). [It should be emphasized that this profound concept applies to all species of animals whether or not they possess cartilaginous or bony skeletons (1,18). In the invertebrates, only

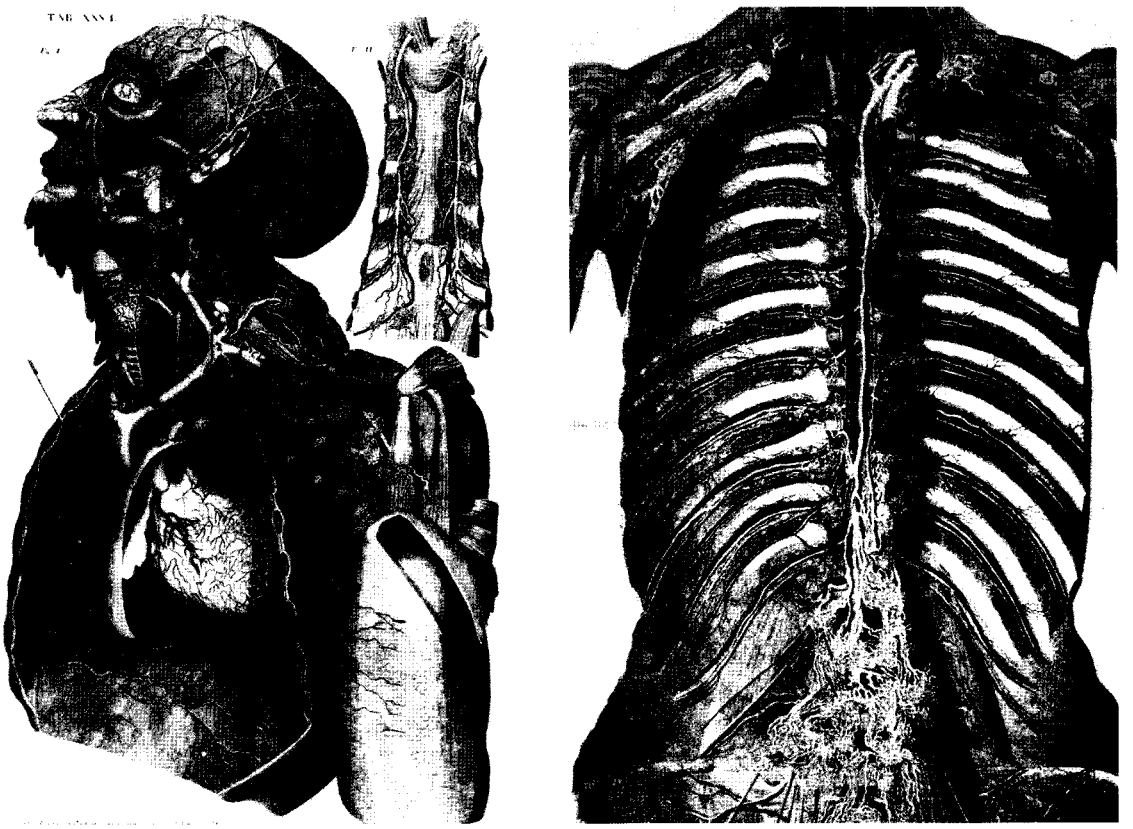

Fig. 5. Mercury injected preserved specimens of dissections of the head and neck and central lymphatics including the thoracic duct by Mascagni of Siena.

lymph circulates (18). In all vertebrates the formation of lymph in the mesenchymal interstices precedes the formation of red blood cells as was shown later by Florence Sabin in 1917 (19) and by Otto Kampmeier (20). As originally deduced by William Harvey, were it not for this vital fluid wherein the red blood cells become suspended before the liver develops, there would be no blood circulation (15).]

In 1898, Ernest Starling of London *(Fig. 6, right)* favored Ludwig's concept of lymph formation via filtration from blood capillaries, after adding colloid osmotic pressure to intravascular hydrostatic pressure to the equation (the well-known filtration hypothesis) (10). These hydraulic principles were expanded upon by numerous physiologists including Landis (21) and in specific organs under pathologic conditions by Rusznyak, Földi, and Szabo *(Fig. 7)* from Hungary (22). It should be emphasized, however, that in the microcirculation as later observed by Krogh, Mackenzie, Zweifach and others, blood flow is relatively constant in arteriolavenous (A-V) thoroughfares but intermittent in A-V capillaries; capillary permeability varies under differing conditions; and A-V sinusoids in the liver, spleen and other organs are lined by highly permeable reticulo-endothelium, instead of flat continuous vascular endothelium (1). Moreover, the respiring parenchymal and interstitial cells located between blood and lymph capillaries synthesize and secrete a variety of low molecular weight proteins

Fig. 6. Karl Ludwig (left) and Ernest Starling (right), co-founders of the filtration hypothesis regarding the origin of lymph from plasma.

whose colloid osmotic effects in lymph remain to be fully appreciated (1,11,15,17).

In 1890, Louis Ranvier of Belgium, after studies in sponges, in *Des Clasmatocytes* (23), showed that Elie Metchnikoff's (of Russia) macrophages excrete material ingested and digested by clasmatosis for the nutritive benefit of adjacent cells. [As opposed to the currently popular term, apoptosis (Greek, *APO* - away + *PTOSIS* - falling) wherein all cell organelles suddenly disintegrate and appear hyaline (24), the term, clasmatosis (Greek, *KLASMA* - fragment + *OSIS* - ation) coined by Ranvier (23) describes the fragmentation of peripheral cell cytoplasm into tiny amorphous globular fragments, which are extruded from the periphery of cells without evidence of nuclear or nucleolar degeneration (1,25). Hence, the clasmatocytes are a class of fragmenting cells whose cytoplasm is shed in the form of tiny globules (1,15,18) *(Fig. 8)*. Alfred Donné in his *Cours de Microscopie*, Paris, Bailliere, 1844 described such tiny globules not attached to lymphocytes in central lymph and called them globulines.]

In 1912, Hal Downey from Minnesota, USA and Franz Weidenreich from Germany in *Ueber die Bildung der Lymphocyten in Lymphdruesen und Milz* (25) described the embryonic and adult origins of lymphocytes and other mononuclear cells in the spleen, nodes and thymus glands by a series of differentiations wherein components of the mesenchymal reticular syncytium in characteristic body regions round up and

Fig. 7. Cecil Drinker (upper left), Mihaly Földi (upper right), Joseph Yoffey (lower left), and F.C. Courtice (lower right) who along with John Kinmonth (see Fig. 11), are the greatest lymphologists of the 20th Century.

Fig. 8. Lymphocyte plasmocytosis in tissue cultures under scanning electron microscopy. Left—a medium sized lymphocyte at 48 hrs with PHA showing an extended uropod presumably in the direction of motion. Note the extrusion of long and short microvilli, some of which break off tiny cytoplasmic globules and extrusion of larger cytoplasmic globules, some of which appear free in the medium especially on the aspect remote from the uropod. Right—2 small lymphocytes at 48 hrs without PHA. One shows a smooth plasmalemma over the nucleus and the uropod extruding microvilli and cytoplasmic globules. The other shows extensive formation of microville and cytoplasmic globules over the entire cytoplasmic surface without apparent nuclear distortion. This form of cytoplasmic fragmentation without nuclear changes was called plasmacytosis *by Louis Ranvier in 1890. Now the terms, cytoplasmic shedding or blabbing are commonly used to describe this process whereby mononuclear cells secrete soluble globulins. Markers indicate 5μ.*

enlarge to become nucleated and nucleolated stem cells for lymphocytes, plasmacytes and other kinds of mononuclear cells. Such lymphopoietic stem cells, in turn, enlarge further and divide by mitosis with increasing nuclear size, chromatin dispersion and cytoplasmic basophilia to create many smaller cells whose nuclear chromatin condenses while peripheral cytoplasm is shed by clasmatosis in the form of tiny globular cell fragments which they termed hyaline bodies. The end result was the formation of myriad small cytoplasm-depleted lymphocytes with increasing nuclear/cytoplasmic ratios, which ultimately migrate from the lymphoid tissues, along with soluble cytoplasmic products of lymphocytes, plasmacytes and other kinds of stromal mononuclear cells, which do not emigrate normally (1). Downey and Weidenreich both emphasized that hyaline body formation and release from larger lymphocytes, plasmacytes, monocytes and reticulum cells is characteristic, but most common in relatively large lymphocytes found in primary lymph follicles, secondary lymph follicles, and periarteriolar lymphatic sheaths *(Fig. 8)*. In 1913, Downey likened the lymphocytic shedding of hyaline bodies without chromomeres to the megakaryocytic shedding of blood platelets (26). Unfortunately, he did not equate hyaline body formation with the secretion of the soluble globulins and/or antibodies in lymph. In 1922, he demonstrated that the sinuses of lymph nodes are lined by flattened mesenchymal cells instead of common vascular or sinusoidal endothelium (27).

In 1915, Justin Jolly (28) of France, following up on initial studies by J. Aug Hammar of Sweden published in 1905, and followed by those reported by David Marine in 1932 and others cited (1,15) described the analogous morphogenesis of lympho-epithelial organs, including the adenoids, tonsils, thymus and avian bursa of Fabricius. Jolly called the bursa a cloacal thymus peculiar to birds and some species of aquatic turtles (29). A common feature appeared to be derivation of the gland epithelium from vestigial gill epithelium, which invaginates into mesenchyme and transforms into endocrine epithelium producing agents which greatly accelerate the local production of lymphocytes (1,15,28), possibly by catalyzing oxidative chain phosphorylations essential to the linkage of high-energy nucleotides into lymphocyte DNA (30).

In 1924, Alexis Carrel (31) in New York City showed that in tissue culture lymphocytes enhance the growth of other cells. He called these cells trephocytes (food or feeding cells – from Gr. *trephein* - to feed and *kytos* - cell).

In 1931, McCutcheon (32) in the USA showed in tissue cultures that small lymphocytes migrate randomly at a velocity up to 40 μM per minute.

In 1939, Florence Sabin (33) demonstrated that plasmacytes shed antibodies to foreign proteins by clasmatosis, like the clasmatocytes of Ranvier shed cytoplasm after ingesting and digesting particulate matter, and the large lymphocytes described by Downey normally shed hyaline bodies. During the 1940s, studies on sequential mononuclear cell reactions to antigens of known composition in regional lymph nodes and in the spleen of mammals revealed that lymph sinus or sinusoidal macrophages (clasmatocytes), plasmacytes, reticulum cells, large lymphocytes and smaller lymphocytes are each sequentially involved over a period of 10-14 days. The sequential hypertrophy and hyperplasia with cytoplasmic globule shedding is away from sinuses or sinusoids toward the arterial supply of each primary or secondary lymph follicle (1,15) *(Fig. 9)*. When evidence accumulated that sensitized small lymphocytes emigrating from the nodes and spleen were capable of destroying foreign cells and microorganisms without intervention of antibodies, it became obvious to pioneer immunologists, such as Peter Medawar in England, Bede Morris of Canberra, and Gustav Nossal of Melbourne that there is normally a continuous flow of endogenous antigens from peripheral cells to intervening organized lymphoid tissues and from there back to the periphery via reactive antibodies and potentially sensitized lymphocytes (1).

[It thus seemed clear that the lymphoid tissues of each animal species not only develop to feed and help regulate growth in remaining body tissues but also to provide immunity. However, in accordance with natural laws of mass/energy conservation, it became apparent that the purpose of immunity is to convert cells and noxious substances entering the internal milieu by any route into substances which can be reutilized for growth, or excreted in harmless forms, along with the metabolic products generated from the gut with each meal and conveyed to the blood circulation in a milky form via the intestinal lacteals discovered by Asellius (1,15).]

In 1942, James Kindred (34) of Virginia, USA found in quantitative studies on healthy well-fed mammals that lymphocytes growing in lymphopoietic tissues, circulating in blood, and migrating in interstices and epithelia normally constitute about 1/4 of total body cells and 1-2% of total body mass in rats and humans. On the basis of mitotic indices in the organized lymphoid tissues, he calculated that the life span of the average lymphocyte is on the order ± 2 days—an average life span later confirmed in 1945 by Andreason and Ottesen (34) by studying the rate of DNA-phosphate turnover in lymphoid organs.

Although it was long known that starvation and other forms of stress rapidly

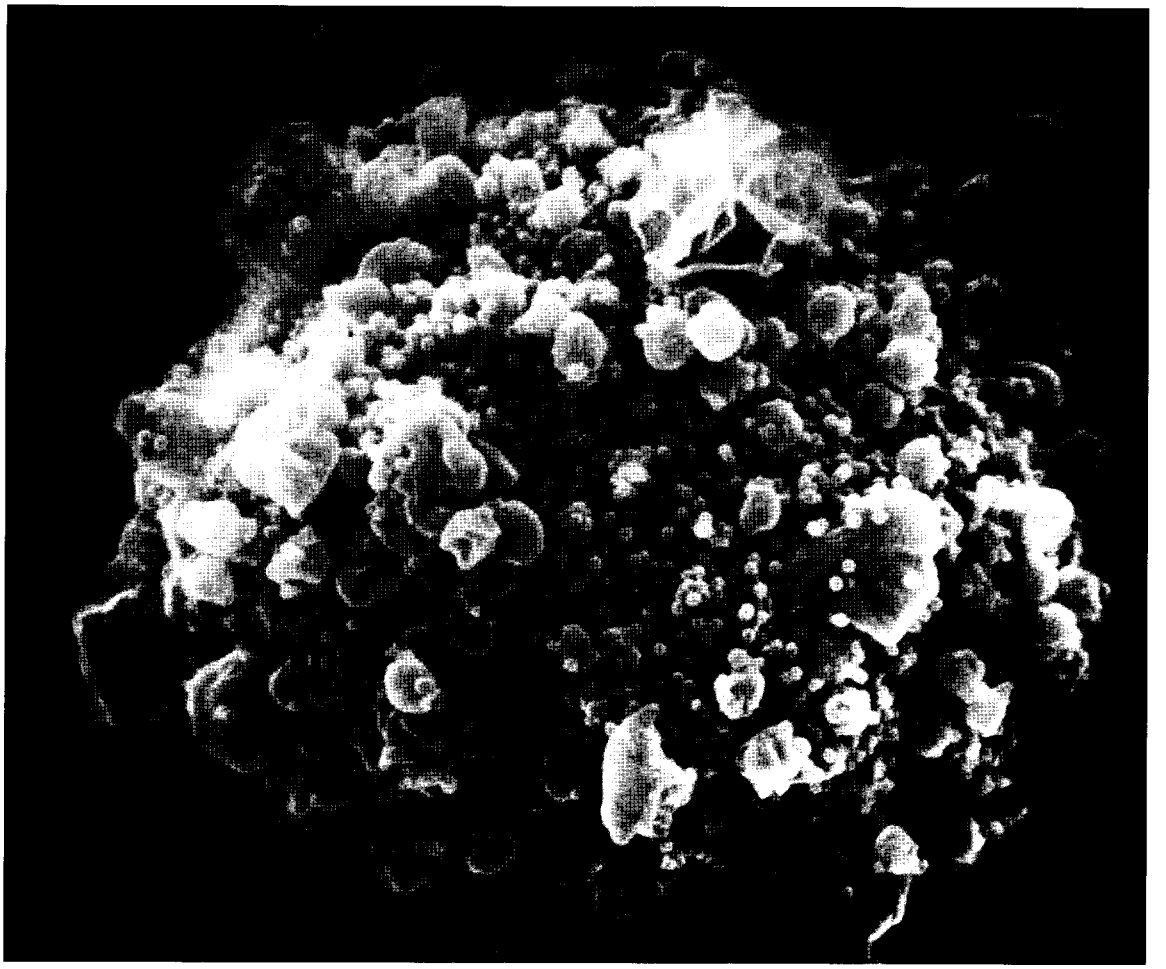

Fig. 9. Globule and HIG-1 shedding from PHA-transformed lymphocytes. In tissue culture, phytohemagglutinin (PHA) characteristically incites SCDL to enlarge, dedifferentiate, and transform into cells resembling LGCL or large follicular lymphoma cells shedding cytoplasmic globules of large, but varying dimensions (arrows). PHA-transformed cells infected with HIV-l and further stimulated with Interleukin-2 derived from cultures of transformed SCDL, in addition, appear to extrude many uniform plasmalemma-encapulated HIV-1 of smaller size from the large globules, as well as from remaining cell surfaces. [Courtesy of Harvey Holmes, National Institutes of Biological Standards and Control (NIBSC), U.K.]

deplete the mass of lymphoid tissues and small lymphocytes in the body, Abraham White and Tom Daugherty in Utah, USA (36) showed that such effects are mediated through the action of adrenal glucocorticoids which increase the rate of cytoplasmic shedding and lymphocytolysis, presumably for the nutritive benefit of other body cells. They were among the first to recognize that cytoplasmic shedding from lymphocytes gives rise to soluble nutritive elements, as well as immunoglobulins.

In 1954, Humble, Jayne and Pulvertaft (36) described lymphocyte *emperipolesis* (Gr. *EM* -in + *PERI* - around + *POLESIS* - wandering) within tissues and cells, more or less at random, and often, just before mitosis in other cells. In 1973, their findings were confirmed by Sherwin and Richters (38), who noted that the *emperipoletic* lymphocytes

sometimes disintegrate within other cells, sometimes induce rapid cytolysis of the other cells, and sometimes pass through without apparent interaction.

In 1959, "Ozzie" Trowell (39) of Australia, but working in England with phosphorous isotope labeling of lymphocyte DNA, deduced that the DNA bound phosphorous from many small emperipoletic lymphocytes is reutilized during mitosis in large germinal center lymphocytes for perpetuating immunologic memory as well as for other purposes.

In 1961 and thereafter, Fichtelius, Diderholm from Scandinavia, Craddock and Bryant from the USA and A.E. Dumont of New York City, one of the founders of the International Society of Lymphology (ISL), demonstrated by isotope labeling that lymphocytes from the thymus and other organized lymphoid tissues donate DNA to enhance functional growth of the neonatal gut and other lymph glands, restore the integrity of liver cells after partial hepatectomy, and enhance the capacity of fibroblasts to heal surgical or traumatic wounds (1,15,40).

During the 1960s and 70s, an astounding mass of experimental and clinical data established that large lymphocytes, not plasmacytes, produce a variety of effective polyclonal antibodies during the normal course of differentiation; and that appropriately sensitized small emperipoletic lymphocytes effectively kill genetically foreign cells and microorganisms which gain access to the milieu intérieur during the course of a customary life span both in vertebrates and invertebrates (18). However, it became evident that all agents which critically reduce the body's mass of organized lymphopoietic tissues and circulating lymphocytes, irrespective of species, produce profound disturbances in homeostasis, especially in the realm of normal tissue growth, as well as in immunity to cells and microorganisms with foreign DNA.

In 1956, Glick, Chang and Jaap (41) reported that testosterone injections into the incubating eggs of Leghorn chickens seven days before hatching promote atrophy of the bursa of Fabricius and impair humoral antibody responses to Salmonella antigens in newly hatched chicks. During the 1960s, comparison of the effects of testosterone bursectomy in Leghorn chick eggs with those of neonatal thymectomy in mammals (42,43) led to the now pervasive belief that the "immune system" is divided into two components—a bursa equivalent, B-cell component derived from bone marrow responsible for the formation of humoral antibodies; and a thymus-derived T-cell component responsible for cellular immunity. The faulty bases for these beliefs include:

1. Surgical bursectomy 10 days before hatching does not impair antibody production in those Leghorn chicks which survive (44).
2. Testosterone injections before hatching induce atrophy of the cervical thymus glands and cloacal bursae of newly hatched chicks (44).
3. Cropsac milk in altricial birds, such as pigeons, and colostrum in mammals are functional equivalents of the bursa of Fabricius in Leghorn chicks (28,29).
4. The avian cloacal bursa has no counterpart in other species (18,28) except some species of turtles, which use these bursae as gills when submerged (18,28-30).
5. Tissue nutrition, regulation of coordinate cell growth and immunity in all species ranging from worms to homo sapiens depends on the sequential development of lymph, lymphatics, organized lymphoid tissues and lymphocytes from the mesenchyme genetically characteristic of species with or without cloacae (18).

In 1965, the ISL launched in Davos and Zurich, Switzerland *(Fig. 10)*, a joint effort on the part of far-seeing radiologists, veteran anatomists and clinicians to understand the workings of the *Lymphatic Apparatus* and *Lympho-Myeloid System* as sequentially defined by Drinker *(Fig. 7, upper left)*, and later Yoffey and Courtice *(Fig. 7, lower left*

Fig. 10. Founders of the ISL at the lst International Congress in Zurich, Switzerland, 1966.

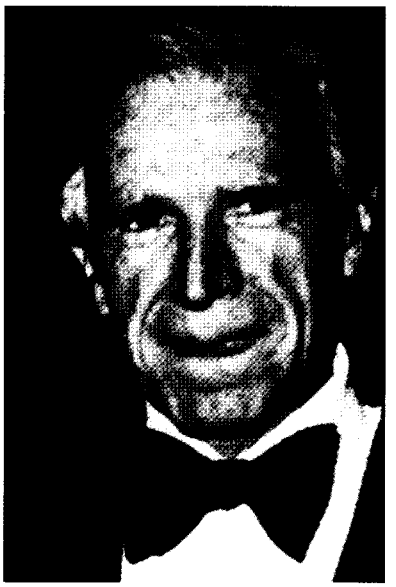

Fig. 11. John Kinmonth, introducer of direct lymphography. See Fig. 7 for other great lymphologists of the 20th Century.

and right) (39) and Courtice's disciple Bede Morris, a brilliant student of Veterinary Science (45). A major impetus to the founding of this Society was the ability to image lymphatics *in vivo* by oil contrast lymphography as introduced by John Kinmonth of London (46) *(Fig. 11)* based on pioneering studies by Servelle of Paris (direct lymphatic puncture) (47) and McMaster of Rockefeller Institute in New York City (vital dye instillation into the skin) (48) for visualizing peripheral lymphatics in health and disease. The fundamental goal was to organize burgeoning knowledge and technology from many nations to help patients afflicted with lymphatic disorders as well as to further understanding of the workings of the lymphatic system.

In 1979, it was discovered that lymphopathic retroviruses were the cause of a form of adult lymphocytic leukemia endemic for

± 300 years in the Kyushu district of Japan (49-51). Shigeo Hino traced the most common source to provirus-infected lymphocytes in maternal milk (49). Using trephones, later called lymphocyte growth stimulating factors, lymphokines and specifically Interleukin-2 derived from phytohemagglutinin (PHA)-stimulated small healthy lymphocytes in tissue culture, Robert Gallo (50) and colleagues at the US National Institutes of Health (NIH) demonstrated the causative retrovirus in mixed lymphocyte cultures. He called the retrovirus "human T-cell leukemia/ lymphoma virus, Type I" (HTLV-I). Later, HTLV-I was found to cause spastic tropical paraparesis in the Caribbean islands and in South America (52). Currently, the spread of HTLV-I, via infected lymphocytes in blood transfusions or shared needles used for injecting drugs, is a serious and burgeoning world health problem (15).

In 1983, Françoise Barré-Sinoussi and Luc Montagnier (53) and colleagues from the Institut Pasteur in Paris, France identified the retroviral cause of the most devastating and pervasive infectious disease the human race has yet encountered. They did so by culturing lymph node cells from homosexual men with immunologically naive umbilical cord lymphocytes stimulated with phytohemagglutinin and supplemental additions of IL- 2. They found that PHA-transformed cord blood lymphocytes had been transfected with a retrovirus which induced the formation and shedding of viral particles whose capsules were formed by the plasmalemma of the transfected dividing cord blood lymphocytes (53). They called the retrovirus Lymphadenopathy Associated Virus (LAV). Subsequently, using the same technology, they discovered a genomically different retrovirus causing a similar but less virulent malady in West Africa. The sickness caused by these lymphocytopathic retro-viruses is now called acquired immuno-deficiency syndrome (AIDS) in America and SIDA in France. The retroviral causes identified at the Institut Pasteur are now identified as human immunodeficiency virus type 1(HIV-1) and HIV-2, respectively.

From a clinical point view, the syndrome, called AIDS, comprises seropositivity and profound lymphocytopenia associated with diffuse tissue wasting, as in "slim disease"; deficient control of tissue growth, as in leukoplakias, superficial keratoses, endocervical dysplasias, lymphomas, Kaposi sarcoma and other malignant neoplasms; as well as impaired resistance to a wide variety of infections, especially tuberculosis, Pneumocystis carinii and cytomegalovirus (15,18,54,55). Such illnesses commonly occur in combination.

Infection in the central nervous system is noteworthy because of the high incidence of cerebral lymphoma (55); and because the blood-brain barrier is normally impermeable to most soluble circulating proteins and leukocytes, except small lymphocytes. The latter normally migrate through the interstices into spinal fluid in small numbers (3,54). The glial cells forming the interstices supporting neurons have a high rate of turnover and have been shown to be transfected with HIV-1 such that they actually shed retrovirions (54,56). Disorderly growth of the glial cells is seldom manifest in the form of gliomas but commonly in the form of AIDS dementia or spinal neuropathies with deficient sensory input and awkward motor coordination (54,55).

The situation in the gut is also noteworthy because the rate of epithelial cell renewal from intestinal crypts, especially in the jejunum, is on the order of ±48 hours (57). Bi-directional small emperipoletic lympho-cyte traffic through the intestinal epithelial cells is common (1,57). Differentially counting 1000 jejunal epithelial cells in thin tissue sections obtained by capsule biopsy from 10 fasting humans under oil immersion microscopy, our group found a mean of 75 ±6 small intraepithelial lymphocytes per 1000 epithelial cells (57). Virtually all (99%) of the intraepithelial lymphocytes were located between the basal lamina and nucleus of each

epithelial cell. More than half (55 %) of the intraepithelial lymphocytes showed progressive degenerative changes, including cytoplasmic swelling, nuclear pyknosis, chromatolysis and debris in progressive stages of dispersion. Barely 1% of the intraepithelial lymphocytes were located between the nucleus and brush border surfaces of the jejunal epithelial cells, and they showed no degenerative changes. Similar studies on bronchial, corneal, and endometrial epithelium obtained by biopsy or excision revealed mean intraepithelial lymphocyte counts of 47±3, 25±10, 35±8 per 1000 epithelial cells differentially counted (1,57). The percentages of degenerating lymphocytes were similar. The significance in terms of lymphocyte migration, disposition and feeding other cells has been described elsewhere (1). It is cogent to add that Jay Levy of San Francisco, USA has demonstrated proviral HIV-1 DNA in the crypt epithelium when villous epithelial cells of the jejunal villi are replaced. This finding, coupled with our observations cited above, may bear on the malabsorption syndrome and "slim disease" frequently seen in persons with AIDS (58).

Barré-Sinoussi (59) went on to emphasize that HIV sickness has been particularly virulent because the retroviral reverse transcriptase(s) are especially error-prone and likely to insert HIV-1 RNA more or less at random into the DNA of dividing lymphocytes. Thus, one or more of the 70-100,000 inherent genes in dividing lymphocytes may become provirus-infected to generate HIV-1 subtypes and clades within subtypes, many of which are insensitive to highly active antiretroviral therapy (HAART) and which make the formulation of specific anti-HIV-1 vaccines extremely challenging if not impossible (54,55,58,59).

In 1993, using immunohistologic methodology and polymerase chain reaction (PCR) technology, Pantaleo (60) and his associates at the NIH and Haase (61) and his colleagues at the University of Minnesota showed that the primary target of HIV-1 infection is in the large dividing lymphocytes in the germinal centers of secondary lymph follicles throughout the body. Moreover, during the latent stages of sickness, large quantities of amorphous HIV-1 RNA are precipitated by gp120 anticapsular antibodies between the large lymphocytes and follicular dendritic cells. Subsequently, Haase and colleagues showed that variably integrated HIV-1 RNA in the DNA of lymphocytes in the spleen, nodes, Peyer patches and blood circulation persist, irrespective of the stage of infection and circulating soluble serum viral RNA concentration, and despite all forms of antiretroviral therapy used to date (61,62). These observations suggest that plasmalemma-encapsulated HIV-1 retrovirions produced by provirus-infected large lymphocytes are efficiently precipitated by anticapsular antibodies emanating from adjacent sensitized cells during the latent stages of HIV sickness—at least until most of the large germinal lymphocytes and their circulating cytoplasm-depleted progeny have been destroyed or incapacitated by the random reverse transcription of HIV-1RNA into their nuclear DNA (54,55,58,59).

Therefore, from a lymphologic point of view, it seems that the random reverse transcription of HIV-1 RNA into the genes of large lymphocytes when the chromosomes segregate and are most unstable; and transport of variably integrated proviral DNA within and between persons via emperipoletic small cytoplasm-depleted lymphocytes derived by nuclear condensation and globulin shedding, promote irreparable damage to the entire lymphopoietic system (54,55,58). The secondary sequelae are progressive failure in homeostasis reflected in deficient tissue nutrition, impaired control of coordinate cell growth, and poor resistance to a variety of infections in multiple combinations, depending on which and how many of some 70 to 100,000 genes have been randomly altered by the reverse transcription of the retroviral RNA during mitosis (54,58,63).

Moreover, it is likely that small

emperipoletic lymphocyte normally numbering 1-4 million/mL in circulating blood, ± 2.5 million/mL in semen; ± 120,000/mL in uterine endocervical secretions; and 1-3 million/mL in maternal colostrum are the most common vectors, which have already spread HIV-1 sickness to more than 50 million persons worldwide via the 0.1 to 1.0 % of infected lymphocytes containing variably integrated HIV-1 RNA in blood, semen, uterine endocervical secretions and maternal milk (54,55).

DISCUSSION

From 1602 to 1981, our preceptors in the science of Lymphology taught us that the sequential development of lymph, lymphatics, organized lymphoid tissues and lymphocytes is essential to circulation, intercellular transfer of molecules required for cell growth and the regulation of effective tissue growth and immunity in all living animals. Since 1981, the advent of HIV-1 sickness, through variable deletion of some of these fundamental physiologic functions, has substantially confirmed these tenets of our preceptors. In addition, the technical advances made by many immunologists have added greatly to our knowledge into the science of lymphology. It is still unclear in 2001 how best this lymphologic knowledge can be used to help sick patients or to advance scientific knowledge for humane purposes. Nonetheless, for those committed to help those afflicted with lymphatic disease, including concerned citizens and others in their home nations, the September 2000 *Lymphology* article by the Wittes on "Ignorance in infectious diseases: The case of AIDS, Kaposi sarcoma and lymphology" (64) is truly lymphspirational! Ignorance, Ignore-ance and complacency are grievous human faults. In the realm of mice, monkeys and men, the role of lymphologists in dispelling ignorance and complacency with respect to lymphopathic retroviral infections would seem crucial worldwide, especially for preventing transfection via needles and to women by all lawful and tolerable means (65).

For those with a passion for DNA, it is rewarding to consider that owing to loss of cytoplasm during normal lymphocyte differentiation, lymph glands and small lymphocytes develop to contain concentrations of DNA 5-20 times greater than that in remaining body tissues and cells (1). Moreover, the lymphocytic DNA appears more labile, malleable, and transportable than that in other tissues or cells, presumably because those 70-100,000 contained genes may be capable of coding singly or in concert for a wide variety of products helpful or harmful to other cells under varying physiologic conditions.

For those with a passion for RNA and its role in altering DNA via reverse transcription, it should prove worthwhile to study the role of plasmacytic RNA in reverse transcribing lymphocytic DNA each time a noxious exogenous or endogenous antigen is encountered in the milieu intérieur (58,66).

For those fascinated by bioenergetics or tissue nutrition, it is useful to consider that the synthesis of nucleosides into phosphate and glucose-linked nucleotides in lymphocytic DNA and RNA requires quanta of energy, mostly derived from the oxidative combustion of absorbed food. How these quanta of energy are reutilized for homeostatic purposes when the linked nucleotides disintegrate is an ergonomic question worthy of continued exploration (1).

For those involved in molecular biology, it might prove timely to consider that lymph enables the intercellular and intravascular transport of water and macromolecules whereas lymphocytes facilitate the transport of DNA from organized lymphoid tissues to other tissues and often directly into the cells in other tissues where cell turnover is relatively rapid (1).

CONCLUSION

We have learned much about lymphology

since 1602, We expect to learn even more in the upcoming ISL Congress in Genoa, Italy where the tradition and teachings of Fabricius, Asellius, Malpighi, Mascagni and Tosatti will be renewed. The science and practice of lymphology remains a fertile field whose surface is barely tilled. With perseverance, however, the harvest should prove bountiful.

Addenda and in Memory of:

John R. Casley-Smith (1936-1997) from Adelaide, Australia and H. Anton Castenholz (1930-1998) from Kassel, Germany, were modern pioneers in lymphology. Their elegant electron microscopic observations on initial lymphatics in sundry parenchymal tissues will eventually pave the way toward a more comprehensive understanding of how mesenchyme (Gr. MESEN - middle; *CHYME* - juice) supports, nourishes and coordinates functions of every living cell in the body. Casley-Smith repeatedly showed that the initial lymphatics serving living parenchymal cells were not lined by epithelium and truly constituted lymph-filled spaces in the amorphous and fibrillary ground substance produced by interstitial mesenchymal cells. Castenholz (67) showed that fragments of fibrillary ground substance extruded into lymphatics. Such observations in adults confirmed and extended the fundamental observations of Sabin, Kampmeier, Ludwig, Heidenhain, Starling, and Bernard, because no lymphatic endothelial barrier or basal lamina is interposed and mesenchymal intercellular matrix not only dissolves but also extrudes into proximal lymphatics lined by endothelium. The mesenchymal intercellular matrix is comprised largely of polymerized low molecular weight glycosaminoglycans, which variably bind interstitial water, depending partly on local oxygen tension, pH, and calcium ion concentration (1). Depolymerization results in the formation of colloidal hydrosols with substantial colloid osmotic effect per mL. (1), At least four cogent physiologic implications follow:

1. Glycosaminoglycans enable peripheral lymph to osmotically retain water under hydrostatic pressure in proximal lymph vessels lined by common vascular epithelium.
2. *In situ* depolymerization of the glycosaminoglycans to create lymph-filled tissue spaces and extrusion of glycosaminoglycans into lymphatics enables peripheral lymph to transport the aqueous, mineral and protein products of each respiring mesenchymal and parenchymal cell, along with elements which diffuse or transude from A-V capillaries for variable processing in regional lymph glands before flowing centrally in the form of central lymph (1). After efficient mixing of the central lymph emanating from all body regions in the gills or lungs to form the plasma in arterial blood, the composite circulates to supply each and every mesenchymal and parenchymal cell in the body according to its needs under a wide variety of physiologic conditions, such that homeostasis is sustained ad infinitum.
3. Regional, as well as general control of such homeostasis in humans, as well as in all species of lower phylogenetic orders, appears to depend on the persistence of functional mesenchyme in the periphery as well as in the reticular stroma of all properly functioning lymphoid and myeloid organs characteristic of species (1,18). Congenital and acquired forms of lymphedema or immunodeficiency are good examples of dysfunction.
4. Studies on angiogenesis (68-70) and liposuction for gathering "pluripotential cells" confirm that mesenchyme persists in the periphery of adults as well as in the mesenchymal reticular stroma of lymphomyeloid organs and the brain.

REFERENCES

1. Shields, JW: *The Trophic Function of Lymphoid Elements*. Thomas, Springfield, 1972.

2. Adelman, HB: *The Embryological Treatises of Hieronymous Fabricius of Aquapendente.* Ithaca, Cornell University Press, 1942.
3. Shields, JW: The Renaissance in Lymphology. In: *Progress in Lymphology.* Weissleder, H V. Bartôs, L. Clodius, et al (Eds.) Prague, Czechoslovak Medical Press, 1981, pp. XIV-XV.
4. Fabricius, H: *De Venarum Ostiolis 1603.* Franklin, KJ (Ed.) Springfield, CC Thomas, 1933.
5. Fulton, JF: *Selected Readings in the History of Physiology.* Springfield, CC Thomas, 1933.
6. Shields, JW: The thymus and the bursa. Blood 33 (1969), 261-262.
7. Harvey, W: *Exercitatio anatomie de motu cordis et sanguinis in animalibus.* Friankfurt, G. Fitzeri, 1628.
8. Harvey, W: *De Generatione Animalium.* London, 1651
9. Aselli, G: *De lactibus sine lacteis venis, quarto vasorum mesaraicorum genere, novo invento.* Mediolani, J.B. Bidellum, 1627.
10. Mayerson, HS: Three centuries of lymphatic history – an outline. Guest lecture at the 2nd Congress of Lymphology, Miami, 1968. Lymphology 2 (1969), 143-152.
11. Shields, JW: Intestinal lymphoid tissue activity during absorption. Amer J Gastroenterol 50 (1968), 30-36.
12. Malpighi, M: *Anatomical Treatise on the Structure of the Viscera*, 1666. World Book Encylopedia, 1966, vol. 13, p. 88.
13. Dameshek, W: William Hewson, thymicologist: Father of Hematology? Blood 21 (1963), 513-516.
14. Hewson, W: Experimental Inquiries into the Properties of Blood, part 3. Printed by T. Cadell in the Strand, 1781.
15. Shields, JW: Lymphspiration: lymph, lymphomania, lymphotrophy and HIV lymphocytopathy: An historical perspective, Lymphology 27 (1994), 21-40.
16. Bernard, C: *Leçons sur les Phénomènes de la Vie Communs aux Animaix et aux Vegetaux.* Paris, Bailliere, 1878.
17. Robin, ED: Claude Bernard, Pioneer of regulatory biology. JAMA 242 (1979), 1283-1284.
18. Shields, JW: The functional evolution of GALT. Lymphology 33 (2000), 47-57.
19. Sabin, FR: Preliminary notes on the differentiation of angioblasts and methods by which they produce blood vessels, blood plasma, and red blood cells as seen in living chicks. Anat Record 13 (1917) 199-204. Springfield, CC Thomas, 1969.
20. Kampmeier, OF: *Evolution and Comparative Morphology of the Lymphatic System.* Springfield, CC Thomas, 1969.
21. Landis, EM: The capillary pressure in frog mesentery as determined by microinjection methods. Am J Physiol 75 (192-26), 548.
22. Ruszynak, I, M Földi, G Szabo: *Lymphatics and Lymph Circulation.* 2nd Edition. Oxford Press, 1967.
23. Ranvier, L: Des clasmatocytes. Compt Rendu Acad Sci. 110 (1890), 165-169.
24. Hengartner, MD: The biochemistry of apoptosis. Nature 407 (2000), 770-776.
25. Downey, H, F Weidenreich: Ueber die Bildung der Lymphocyten in Lymphdruesen und Milz Arch Mikr Entw Mech 80 (1912), 306-395.
26. Downey, H: The origin of blood platelets. Folia Haematol 1 (1913), 25-58.
27. Downey, H: The structure and origin of lymph sinuses of mammalian lymph nodes and their relation to endothelium and reticulum. Haematology 3 (1922), 431-468.
28. Jolly, J: La bourse de Fabricius et les organes lymphotheliaux. Archs Anat Microsc16 (1915), 363-547.
29. Shields, JW, DR Dickson, W Abbott, et al: Thymic, bursal and lymphoreticular evolution. Developmental Comparat Immunol 3 (1979), 5-22.
30. Shields, JW: Bursal dissections and gill pouch hormones Nature 259 (1976), 373-376.
31. Carrel, A: Leukocyte trephones. JAMA 82 (1924), 255-258.
32. McCutcheon, M. Studies on the locomotion of lymphocytes III. The rate of locomotion of human lymphocytes in vitro. Amer J Physiol 69 (1931), 279-282,
33. Sabin, FR: Cellular reactions to a dye-protein with a concept of the mechanism of antibody formation. J Exper Med. 70 (1939), 67-82.
34. Kindred, J: A quantitative study of the hemopoietic organs of young albino rats. Amer J Anat 3, 67 (1940), 99-149 and 71 (1942), 207-243.
35. Andreason, E, J Ottesen: Studies on the lymphocyte production. Investigations on the nucleic acid turnover in the lymphoid organs, Acta Physiol Scandinav 10 (1945), 258-270.
36. White, A, TF Dougherty: The role of lymphocytes in normal and immune globulin production and the mode of release of globulin from lymphocytes. Ann. NY Acad. Sci. 46 (1946), 859-882.
37. Humble, JG, WHW Jayne, RJ Pulvertaft: Biological action between lymphocytes and other cells. Brit J Haematol 2 (1956), 283-294.
38. Sherwin, RP, A Richters: Pathobiology of lymphocyte interactions, Pathology Annual 8 (1973), 379-406.

39. Trowell, OA. Reutilization of lymphocytes in lymphopoiesis. J Biophys Cytol 3 (1957), 317-318.
40. Yoffey, JM, FC Courtice. *Lymphatics, Lymph and the Lymphomyeloid Complex*. London, Academic Press, 1970.
41. Glick, B, TS Chang, RG Jaap: The bursa of Fabricius and antibody production. Poultry Science 35 (1956), 224-225.
42. Warner, NL, A Szenberg, FM Burnet: Immunological role of different lymphoid organs in chickens. Austral J Exp Med Sci 40 (1962), 373-387.
43. Cooper, MD, AR Lawton: The development of the immune system. Scientific American 231, (1974)
44. Fitzsimmonsm RC, EMF Garrod, I Garret: Immunological responses following early embryonal surgical bursectomy. Cell Immunol 9 (1973), 377-383.
45. Courtice, FC: In Memoriam: Bede Morris, PhD, DVM—Lymphologist Extraordinaire, 1927-1988. Lymphology 21 (1988), 198-202.
46. Kinmonth, JB: Lymphangiology in man. A method of outlining lymphatic trunks at operation. Clinical Science 11 (1952) 13-20.
47. Servelle, M: *Pathologie Vasculaire*. Masson, Paris, 1952, p. 299
48. McMaster, PD: Lymphatic participation in cutaneous phenomena. The Harvey Lectures 1941-1942, p. 227-268.
49. Hino, S: HTLV-I infection: Milk-borne infection as the major pathway for peculiar endemia. Asian J. Med. 32 (1989), 239-245.
50. Wong-Stall, F, RC Gallo: The family of human T-lymphotropic leukemia viruses: HTLV-I as the cause of adult T cell leukemia and HTLV-III as the cause of acquired immunodeficiency syndrome. Blood 65 (1985), 253-263.
51. Shields, JW: Lymphocytostatic prevention of ATL and AIDS. In: *Progress in Lymphology XII*. Nishi, M, S Uchino, S Yabuki (Eds.), 1990, pp. 521-522.
52. Gessain, A, F Barin, JC Vernant et al: Antibodies to T-lymphotropic virus Type I in patients with tropical spastic paraparesis, Lancet ii (1985), 4907-4910.
53. Barré-Sinoussi, F, JC Chermann, F Rey, et al: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220 (1983), 868-871.
54. Shields, JW: The XI AIDS Conference and HIV-1 Lymphopathy. [Special Communication] Lymphology 30 (1997), 137-154.
55. Cox, FD: *The AIDS Booklet*. McGraw-Hill Higher Education, Sixth Edition, 2000.
56. Levy, JA, J Shimabukoro, H Hollander, et al: Isolation of AIDS-associated retroviruses from and brain of patients with neurological symptoms. Lancet ii (1985), 586-568.
57. Shields, JW, RC Touchon, DR Dickson: Quantitative studies on small lymphocyte disposition in epithelial cells. Amer J Pathol 54 (1969), 129-145.
58. Shields, JW.: Ignorance in AIDS, KS and septicemias. [Commentary] Lymphology 33 (2000), 172-176.
59. Barré-Sinoussi, F: HIV as the cause of AIDS. Lancet 348 (1996), 31-35.
60. Pantaleo, G, G Grasiosi, JF Demarest, et al: HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of the disease. Nature 363 (1993), 355-358.
61. Embretson, J, M Zupanic, JL Ribas, et al: Massive covert infection of helper T-lymphocytes and macrophages during the incubation period of AIDS. Nature 362 (1993), 359-362.
62. Haase, AT, K Henry, M Zupanic, et al: Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science 274 (1996), 985-989.
63. Haase, AT, TW Schacker: Potential for transmission of HIV-1 despite highly active antretroviral therapy. N. Engl J Med 339 (1999), 1846-1848.
64. Witte, MH, CL Witte: Ignorance in infectious diseases: The case of AIDS, Kaposi sarcoma and lymphology. Lymphology 33 (2000), 95-121.
65. Shields, JW: A rational basis for safe sexual conduct in preventing the spread of AIDS Lymphology 26 (1993), 1-3.
66. Shields, JW: Cytoclasmosis, reverse transcription and DNA reutilization in immunity. In: *Progress in Lymphology*. Weissleder, H, V Bartôs, L Clodius, et al. (Eds.) Prague, Czechoslovak Medical Press, 1981, pp139-141.
67. Castenholz, A: Functional microanatomy of initial lymphatics with special consideration of the extracellular matrix. Lymphology 33 (1998), 101-118.
68. Shields, JW: Normal and tumor angiogenesis in relation to flow. Lymphology 32 (1999), 77-80.
69. Wilting, J, M Schneider, M Papoutsi, et al: An avian model for studies of embryonic lymphangiogenesis. Lymphology 33 (2000), 81-94.
70. Shields, JW: Lymph and lymphangiogesisis. Functional aspects. [Editorial Commentary] Lymphology 33 (2000). 77-80.

Jack W. Shields, M.D.
1950 Las Tunas Road
Santa Barbara, CA 93103 USA