Lymphology 22 (1989) 154-156

EDITORIAL

MILESTONES IN LYMPHOLOGY*

For two centuries or more after the discovery of the lacteals in 1627, the relationship between the lymphatic and blood circulations remained very much an enigma. It was not until the middle of the 19th century, a time of great activity in the medical sciences in Europe when quantitative measurement was being added to the qualitative observations of physiological phenomena, that great advances were made in the clarification of this problem. One of the leaders in this field was the eminent professor at the Physiological Institute in Leipzig, Carl Ludwig. Born in 1816, he was at the height of his career as a medical scientist. His practical experiments in the laboratory formed the basis of his concepts of physiological mechanisms which opened a new chapter into the study of the lymphatic system. First, his rational thinking and persistent desire for measurement led to his filtration theory of lymph formation. According to this theory, the lymphatic vessels returned to the bloodstream the fluid that had filtered from the blood plasma through the capillary wall under a head of hydrostatic pressure. Any measurement of lymph flow would determine thereby the extent of this filtration process and so have a clear physiological meaning.

However, it was at this time not easy to measure lymph flow; nor has such a measurement ever been easy. This did not daunt Ludwig and his pupils. The second spectacular achievement coming from his laboratory was the cannulation of small subcutaneous lymphatic vessels in the leg of the dog, a remarkable instance of manual dexterity considering the anesthesia available. A glass cannula was inserted into a lymphatic vessel draining the hind foot of the dog anesthetized with opium. Under these conditions, no lymph actually flowed from the cannula except when the foot was massaged. When the foot was injured by immersion in hot water, however, lymph did flow continuously from the cannula: "Lymph immediately begins to drop most beautifully from the cannula. Thus it may be regarded as established that in inflammation as in venous hyperaemia, a considerable increase takes place in the transudation from the blood vessels, leading first to an increase in the lymph stream and only when the lymphatics are no longer adequate to carry off the transudation, to a swelling of the affected part" (1). This citation is a striking example of the early quantification of lymph flow and brilliant insight into the function of the lymphatic vasculature.

Ludwig's concept of lymph formation was certainly a milestone in the progress of our knowledge of the lymph circulation but it was not universally accepted. Heidenhain's secretion theory gained support until, in the late 1890's, the balance of opinion once again began to turn back to Ludwig's concepts, albeit in a modified form. The man who was to bring a new concept to lymph formation was a young physiologist from London, Ernest Starling. Starling became interested in physiology while a medical student at Guy's Hospital and in 1885 at the age of 19 he was sent by his professor to Heidelberg to work under the German physiologist, Wil-

Delivered as part of a symposium on Methods to Assess and Quantity Lymphatic Flow--XIIth International Congress of Lymphology, Kyoto, Japan, September 2, 1989.

Fig. 1. Ernest Starling during a visit to Germany as a young man (age 20 years).

helm Kühne. *Fig. 1* shows the young Ernest Starling when he was in Germany at that time.

On returning to London, Starling resumed his medical training, qualifying in medicine at Guy's Hospital. His enthusiasm for the experimental method in medical research, however, led him to move to University College in 1890 where the facilities for research were better. He was so influenced by the new scientific approach to medicine in Germany that he returned there in 1892, at the age of 26, to work in Breslau with Heidenhain on lymph formation. At the time, the results of their experiments were interpreted as supporting the secretion theory. However, on returning to London, Starling repeated the experiments, made further measurements and in 1896 published a classical paper which supported Ludwig's filtration theory with an important modification implicating the plasma proteins in the process. The word "protein" had first been introduced by Mülder in 1838, but it was in the second half of the 19th century that the physical properties of these macromolecules were clarified. Starling's keen perception of the osmotic forces that the plasma proteins might exert led him to the concept of their role in the maintenance of fluid balance across a semipermeable membrane. His ideas have, for almost 100 years now, dominated thinking on lymph formation and served as a strong stimulus to anyone interested in the quantification of lymph flow.

The stage was set for physiologists to answer afresh the question, "How meaningful is a measure of the lymph flow from any region of the body?" World War I intervened, but in the 1920's and 1930's this question was vigorously pursued by Cecil Drinker and his pupils at Harvard. In 1926, Drinker went to Copenhagen to work with August Krogh. Drinker later wrote, "We became interested in lymphatics and lymph as a result of experiments upon the frog. In the amphibia the lymphatic system seems to be part of the general circulatory apparatus. Fluid leaving blood capillaries moves constantly into lymphatics or lymph spaces and returns rapidly to the blood" (2). On returning to Harvard, Drinker turned his attention to the mammalian lymphatics using glass cannulae to collect lymph and measure lymph flow. His experiments led him to the view that there is a continuous one-way extravascular circulation of plasma protein. By collecting lymph coming from a tissue and by estimating the protein concentration in that lymph, a measure of the amount of protein escaping from the blood plasma was obtained. The collection of lymph from a glass cannula, with the necessary periodic introduction of powdered heparin into the cannula to prevent clotting, was a laborious procedure and could really be used only in anesthetized animals. Nevertheless, Drinker's accurate measurements supported Starling's hypothesis and further stimulated many others to explore the function of the plasma proteins in lymph formation.

Drinker, of course, as did others up to the middle of the present century, made unsuccessful attempts to produce a chronic lymphatic fistula from which lymph would flow continuously over long periods

of time in a conscious animal. With the materials available, the main stumbling block was the clotting of the lymph in the cannula with cessation of lymph flow. After World War II, however, the advent of plastic tubing largely solved this problem. Bollman and his colleagues at the Mayo Clinic in 1948 described their new classical technique for the long-term collection of lymph from the liver, small intestine and thoracic duct of the rat, using polyethylene tubing. This technique revolutionized the quantification of lymph over long periods of time. Though recognizing the great resurgence of interest in lymph flow generated by Drinker's techniques, lymphologists discarded their glass cannulae in the early 1950's in favor of the new plastic tubing which retarded clot formation and made chronic lymphatic fistula experimentally possible.

Another key milestone in our advancing knowledge of the function of the lymphatic system during the past 100 years concerned the cells in lymph. In 1956, J.M. Yoffey wrote, "Granting the assumption that large numbers of lymphocytes are daily entering the blood via the thoracic duct, the question arises as to whether these are newly formed cells, or whether they are cells which have entered the lymph from the blood stream. Nothing is known of any possible function that such a circulation of lymphocytes might subserve" (3). But, technologically the stage was set for a solution to this problem. Only 3 years later, in 1959, J.L. Gowans in Oxford, using the Bollman rat preparation and new methodology for labeling lymphocytes, demonstrated the recirculation of these cells in lymph nodes. These experiments dramatically changed our thinking on the migration of lymphocytes throughout the body and pointed to the value of the quantification of lymph flow in the determination of the extent of trafficking of lymphocytes when the body is challenged by an antigen or an allograft.

As Drinker led the way in determining the extent of protein movement throughout the body fluids following Starling's enunciation of his concept of the role of these macromolecules, so Bede Morris was an outstanding leader in determining the extent of lymphocyte trafficking in the body. Morris, who was so tragically killed in a motor car accident near Paris in 1988 at the age of 61 (see *Lymphology* 21:198, 1988), used the sheep as his experimental animal. Working in the John Curtin School of Medical Research at the Australian National University in Canberra where he was my colleague for many years, Morris exploited the use of polyethylene cannulae and other modern advances in technology to quantify the trafficking of lymphocytes in the development of the immune system in the fetus and in the development of immunity when a lymph node is challenged with an antigen. A superb experimental surgeon and lymphologist, he was certainly a master in his field of endeavor and ranks with those other great lymphologists I have mentioned.

REFERENCES

1. Cohnheim, J: *Lectures on General Pathology* (translated from 2nd German edition by Alexander McKee), Vol. I, The New Sydenham Society, London (1889), 264-265.
2. Drinker, CK, ME Field: *Lymphatics, Lymph and Lymphoid Tissue*, Bailiere, Tindall and Cox, London (1933), xiii.
3. Yoffey, JM, FC Courtice: *Lymphatics, Lymph and Lymphoid Tissue*, Edward Arnold, London (1956), 344.

Dr. F.C. Courtice
3 Bonney Close
St. Ives, NSW 2075
AUSTRALIA