MUSEUM SPECIMENS OF THE MAIN SUPERFICIAL AND DEEP LYMPHATICS OF THE LEG IN MAN

by

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Historical introduction

Most anatomical structures were known from earliest times but the lymphatic system was only "discovered" as late as the 17th century; the vessels had almost certainly been observed by early anatomists but had been classed as nerves. Gaspare Aselli (1581–1626) of Cremona is usually credited with the discovery of the "lacteals", the lymph vessels of the small intestine, which differ from other lymph vessels only in regard to their function of fat absorption. His book De lactibus sive lacteis venis, the first anatomical work to have coloured illustrations, was published in 1627 but he had detected the vessels on 23rd July 1622 when dissecting a dog in order to demonstrate the movements of the diaphragm. He noticed the vessels ramifying in the mesentery and thought them to be nerves until, on continuing the dissection, he found the true nerves. Taking a scalpel he pricked one of the vessels and a milky fluid, the chyle, gushed out. On repeating the experiment the following day he was not so fortunate; but a third attempt on a well-fed animal confirmed the presence of the structures and he later demonstrated them in cats, sheep, cows, goats and horses.

Jean Pecquet (1622–1674) discovered the receptaculum chyli in 1647 and published his findings in 1651 with the title Experimenta nova anatomica quibus incognitum hactenus chyli receptaculum, et ab eo per thoracem in ramos usque subclavios vasa lactae deteguntur. Ejusdem dissertatio anatomica de circulatione sanguinis et chyli motu. He introduces his dissertation with the following remarks:

"The enrichment of the Anatomical Commonwealth by the Milkie Veins was the finding, not feigning, of the famous Asellius. But that he believes, with the Antients, that the Liver is the source of blood and that the Milkie Veins have their confluence to it, This is (O Reader, if thou canst behold with thy eyes) his Errour. Nevertheless beware in the least thou stain not the fame of so gallant a man. "Tis not a Little thou owest him who first search'd out the unknown turnings of the Chyle, and by whose favour thou needs not to walk in darkness." (English edition, London, 1653.)

He gives an account of his investigations as follows (p. 7):

"After I had some years ago, by cutting up of Dead Bodies, acquir'd a dumb (I may say) and cold Knowledge, I resolved to squeeze forth true knowledge from the Harmonie yea of twenty living Creatures. . . . Therefore having cloven asunder the Thorax of a Great Hound, I begun my view of the contained parts without delay; I pluckt out the heart, having cut asunder those Vessels wherewith it was tied to the rest of the body. . . . From the Subclavian Branches unto the Pericardium within the Vein there settled down a very white Liquor, most like the Chyle spread abroad in the Mesentery; so that they being compared together, both their colour, smell, tast and consistence shew there was no difference betwixt them " (p. 9).

For his second experiment he had an interested audience. Unfortunately, the dog was so lean that the lacteals were empty; and though
he protested to the onlookers that "the Pipes of the Lacteal Veins were most slender and onely remarkable by the whiteness of the Liquor they convey" (p. 13), they were obviously unbelieving of his statements. "In vain," he says, "is the Chanel sought, when the fulness of the Fountain faileth. Neither is Chyle procreated by fasting, neither is meat presently after the eating thereof converted into that nourishing substance" (p. 14). Having nourished his dog, he was more successful and "then the Spectators contempt repenting at the miracle, by their encouragement it was imposed on me to search out whether any continued Conduit of the Chyle did go to the Head, or was derived to the Fore-limbs" (p. 17). A further experiment enabled him to find the receptacle the existence of which he had long suspected. He describes the confirmation of his theory as follows:

"At last I cut with a sparing knife all the hiding tunicles; Such a Bladder I espied, not divorced from the Aorta, but as it were received into its protection, under the Coeliack stem and Emulgent branches, neither altogether lurking, nor altogether appearing. So at last was laid open that most desired storehouse of the Chyle, and that Receptacle searched out by my so much labour" (p. 27).

It was Olaf Rudbeck (1630–1702), a student at Uppsala, who first recognized a general system of lymphatic vessels in 1650, priority in this being disputed by no less a scientist than Bartholin. Two years later Rudbeck had the honour of demonstrating these structures to Queen Christina and her court, then in Uppsala, where he succeeded to the Chair of Anatomy and Botany. His description of the vessels, consisting of only 36 pages, published in 1653, bears the title Nova Exercitatio Anatomica, exhibens ductus hepaticos aquosos et vasa glandulorum serosa and is amazingly precise. He even describes the valves in the vessels, a fact later forgotten and "discovered" again by Friedrich Ruysch in 1721.

In the 18th century, research into the function and distribution of the lymphatic vessels was attended by bitter dissension and jealousy among the anatomists. William Hunter was moved in 1762 to publish in his Medical Commentaries a justification of his claim to priority in stating that the lymphatics were absorbent vessels for which the Monros desired the credit. John Hunter gave positive support to his brother's views and included them in the catalogue of his museum. William Hewson published his findings about the lymphatic system in Birds, Fish and the animals called Amphibious in 1774 (Experimental Enquiries); and William Cumberland Cruikshank produced his Anatomy of the Absorbing Vessels of the Human Body in 1786. Two years previously, John Sheldon completed the first part of his work on the absorbent system, the only part that was ever published. (The History of the Absorbent System; Part the First, containing the Chylography.) John Hunter was a subscriber to the cost of production and he is mentioned in the text as follows:

"Some years ago, when from observing several phenomena in the human body,
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the idea first suggested itself to me, that the earth of the bone was continually changing, by the deposition of new matter, and absorption of the old; I had a conversation on this subject with Mr. J. Hunter, who informed me that he had fed himself with madder. I immediately asked him, whether he had observed that the earth of his urine was tinged by the madder; to which he answered, that the earth attracted by the sides of the vessel, in which the urine was made, was of a red colour " (p. 31).

In order to trace the absorbents Sheldon mentions one method which he believed had been practised by Malpighi:

" If we steep the parts in water, and change the water as long as it is tinged by the blood being soaked out of the blood vessels, suffering them afterwards to remain in the same water till putrefaction begins; the fixed air that is let loose by this process will get into the absorbents, they will be easily seen, may be opened, and injected with quicksilver " (p. 9).

He remarks, however, that this method does not answer so well in the human subject as in quadrupeds. Nevertheless he does describe the technique he found practicable:

" In injecting the lymphatic vessels of the superior and inferior extremities of the human body, it is useful to raise the part where the pipe is inserted higher than the other end of the limb, and to make the assistant press with his hands along the skin, in the course of the vessels, which will favour the progress of the injection. When the vessels are sufficiently filled, (which the anatomist determines by the turgescency of them, evident to the sight and touch, and by the resistance made by the vein, and the quicksilver in the tube) the assistant passes a thread by means of a needle, if necessary, under the vessel, and ties it above the puncture, before the anatomist withdraws the injecting pipe " (p. 11).

The book was a most learned treatise that corrected many of the errors of earlier writers on the subject, and it is to be regretted that it was never completed. Antonio Scarpa, then Professor of Anatomy in Pavia, prepared two of the illustrations. Jesse Foot praised it especially for the fact that Sheldon made no secret of his methods for, he says:

" The Progress of the science has been much impeded by a mystery among anatomists, who have generally concealed the composition of their injections, and their methods of dissecting, injecting and preparing the different parts, a mystery unworthy the character of a philosopher."

The "mysteries" were greatly clarified by Thomas Pole (1754–1829), M.D., a Member of the Company of Surgeons, who in 1790 published his excellent work The Anatomical Instructor or, an Illustration of the modern and most approved methods of preparing and preserving the different parts of the Human Body and of Quadrupeds, by Injection, Corrosion, Maceration, Distention, Articulation, Modelling etc. Of the lymphatics he observes:

" They are small delicate transparent vessels, appear knotted, or irregular, from the abundance of their valves, and are found arising from every part of the body. In order to discover these vessels, make an incision in the cutis, and remove a part of it as far as the cellular membrane, where they arise plentifully; yet from their transparency, may so elude the eye, as to require a magnifying glass to discover them " (p. 95).

" The subjects most favourable for injecting, are those who have died anasarcous, as in such the lymphatics are somewhat enlarged, and more evident. This is one of the most delicate preparations, requiring the greatest dexterity of any part of experimental anatomy. . . . "

When the difficulty of making these mercury injections is considered, it is astonishing to find how complete and accurate many of the prepara-
tions were, according to the illustrations included in works on the subject. One of the finest of these is the *Prodrome d'un ouvrage sur le système des vaisseaux lymphatiques* published in 1784 by Paolo Mascagni (1752–1815), Professor of Anatomy in Siena, probably the greatest exponent of all time of the art of injecting lymphatics with mercury. Unfortunately, none of the specimens made by Anton Nuck (1650–1692) have survived. By mixing the mercury with lead and zinc he succeeded in obtaining a solid substance in the vessels and is said to have prepared a specimen of the complete lymphatic system in a man.

Vincenz Fohmann (1794–1833) distinguished himself in this field by devising a technique for demonstrating lymphatic vessels too small for the insertion of a cannula. His * Mémoire sur les Vaisseaux Lymphatiques de la Peau, des Membranes Muqueuses Sèreuses, du Tissue Nerveux et Musculaire* was published in Liège in the year of his death.

More recently the only significant contribution to the techniques of preparing specimens to demonstrate the distribution of the lymphatic vessels was made by Dimitru Gerota (1867–1939) of Bucharest. He made use of the oil paint "Prussian Blue" and similar materials in other colours apparently with great success, but the relatively few preparations to be found using this method for demonstrating the lymphatic vessels seem to indicate that it had a limited application.

The use of the method employed in lymphangiography for locating the vessels, combined with the injection of neoprene latex, has greatly facilitated the preparation of museum specimens of the lymphatics. Latex is the ideal material for injecting lymphatics as it flows the whole length of the leg without risk of rupturing the vessels and sets within minutes of injection (in small vessels) into a tough elastic solid.

**Technique**

When possible a leg which is to be amputated should be selected. About 2 ml. of an 11 per cent aqueous solution of patent blue (Kinmonth *et al.*, 1955), sterilized by autoclaving, is injected subcutaneously into the interdigital webs of the toes, about five minutes before the circulation of the leg is arrested. This allows enough time for the dye to be absorbed by the lymphatics.

Lymphatics, large enough to be cannulated, should be located within two hours of amputation. If cannulation is delayed beyond this time, the dye diffuses into the surrounding tissue, so that the vessels are no longer easily found. Superficial lymphatics are found by making a transverse incision across the dorsum of the foot; the deep trunk is situated posterior to the medial malleolus and runs alongside the posterior tibial artery and nerve.

To facilitate cannulation of the lymphatic, a short length of it must be isolated from the tissue in which it lies, otherwise the needle is likely to penetrate the adhering tissue instead of entering the lumen of the
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vessel. The apparatus for cannulation consists of a fine needle attached to about two feet of slender polythene tube. The relatively long piece of tube makes it possible to inject latex with a hypodermic syringe without risk of disturbing the needle. This apparatus can be made cheaply by cementing a 27 gauge dental needle, with several applications of poly-styrene cement, to a length of polythene tube. Before use the tube should be filled with very dilute ammonia solution. Immediately the needle has been tied into the lymphatic, a little dilute ammonia is injected to confirm that the needle is correctly inserted. There should be no escape of fluid round the point of insertion.

Fig. 1. (a) This leg was injected and partially dissected by Dr. J. Pflug at the Royal Postgraduate Medical School, London. The work was completed at the Royal College of Surgeons of England. Superficial and deep lymphatics have been filled with latex. Specimen No. S147 in the museum of the R.C.S. (b) The photograph shows a lymphatic and vein, both filled with latex, exposed by cutting a window through the skin of the leg. The superficial lymphatics were injected without the aid of patent blue. Specimen No. S148.

A plastic 2 ml. syringe filled with neoprene latex is connected to the polythene tube by means of a hypodermic needle which fits tightly into the tube. Latex is injected at the rate of about 1 ml. per minute. A slower rate would increase the risk of the latex setting prematurely in the vessels before the injection was completed.

Loss of latex from cut ends of lymphatics at the site of amputation is checked by applying a pad of cotton wool soaked in 10 per cent acetic acid, which sets the latex instantly. This injection is continued until the latex extravasates, in order to distend the lymphatics to their maximum
capacity. Extravasation is indicated by a sudden drop in the pressure necessary to operate the syringe. Although excessive extravasation should be avoided, this is not a serious matter, as the surplus latex can later be dissected away without damage to the surrounding structures. Lymphatics terminate on the surface of lymph glands. Latex will not pass through the substance of the glands to fill the efferent vessels, but the injection can be continued by plunging a needle, attached directly to the hypodermic syringe, into the centre of the gland where the efferent vessels arise.

It is desirable when a specimen of the lymphatics is being prepared, to inject the veins with latex also. This is done by tying one or more small polythene cannulae into veins of the foot. Latex is injected by means of the standard injection apparatus (see Tompsett, 1956) until it escapes at the site of amputation. The cut veins are then clamped, and the injection continued to produce a firm distension of all the veins. Finally the leg is fixed by perfusion of the arteries with 4 per cent formalin. If a small amount of acetic acid is added to the formalin the setting of the latex in the veins is hastened.

The tough rubbery consistency of latex makes dissection and cleaning of injected lymphatics and veins comparatively easy. Before mounting, the dissection is coated with gelatin (see Tompsett, 1956) to stick loose ends of connective tissue to the surface. It is then left in 4 per cent formalin solution, until the fluid is no longer stained with patent blue, which may continue to diffuse out of the specimen for six to 12 months. Figure 1 (a) shows a dissection of a leg injected by this method.

If only post-mortem material is available, similar specimens can be prepared, provided that the lymphatics can be located without the help of patent blue. To facilitate the search for lymphatics suitable for cannulation, a leg should be taken from a subject who, in life, might have been described as "nothing more than skin and bones", as the presence of fat greatly increases the difficulties. Satisfactory lymphatic injection can be done at least a week after death, provided that the cadaver has been stored under refrigeration; but the leg must be restored to room temperature before the latex injection is started, as latex may set prematurely under very cold conditions. Fixed material cannot be used for this work, as fixation rapidly destroys the elasticity and strength of lymphatics.

Some difficulty may at first be encountered in distinguishing lymphatics from small veins, arteries and even nerves. Lymphatics are recognized by three features, viz. the uniform diameter of large vessels which is about 1 mm.; their transparency and lack of colour; and their infrequent branching. They are distinguished from small arteries and nerves because both these are more opaque.
REFERENCES


"OBSERVABLES" AT THE ROYAL COLLEGE OF SURGEONS

53. The Frankau Cup

The Frankau Cup was bequeathed to the College by the late Sir Claude Frankau, F.R.C.S. (1883–1967), who was a member of the Court of Examiners for eight years. The cup was presented to Sir Claude’s father on his retirement in 1924 as Deputy Treasurer of St. George’s Hospital, London.

This elegant two-handled cup is described as follows:

“George III silver half-fluted cup and cover with applied medallions of classical figures, London 1783, 61 oz., with the inscription ‘F. I. Frankau, Deputy Treasurer, St. George’s Hospital 1914–1924 from the House Committee and Honorary Medical and Surgical Staff’.”