



This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

- + *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.
- + *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.
- + *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.
- + *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at <http://books.google.com/>

LANE MEDICAL LIBRARY STAMFORD

2 45 0179 4479

THE JOHNS HOPKINS HOSPITAL REPORTS
MONOGRAPHS. NEW SERIES No. V

THE ORIGIN AND DEVELOPMENT OF
THE LYMPHATIC SYSTEM

BY
FLORENCE R. SABIN

(From the Anatomical Laboratory of The Johns Hopkins University, Baltimore)

LANE LIBRARY

BALTIMORE
THE JOHNS HOPKINS PRESS
1913

(Copyright, 1913, by The Johns Hopkins Press)

E197
S11
1913

LANE

MEDICAL



LIBRARY

ANTOINE BOREL FUND

LANE LIBRARY

CONTENTS.

	PAGE
I. Introduction	1
1. Morphology of the vascular system, the angioblast	1
2. Morphology of the lymphatic system.....	4
II. Historical	4
1. Vasa serosa	4
2. Lymphatics with open mouths.....	4
3. Discovery of endothelium.....	6
III. Embryology	7
1. Introduction	7
2. Lymphatics as dilated tissue spaces.....	8
3. Analysis of Budge's work.....	8
4. Statement of the three arguments for the origin of lymphatics from the veins.....	10
A. Growth of lymphatic capillaries by sprouting.....	10
B. Lymphatic and non-lymphatic zones.....	18
C. Direct budding of the lymphatic vessels from the veins	21
IV. Primary lymphatic system in different forms	24
1. Primary lymphatic sacs in the pig.....	24
A. General summary of the sacs.....	24
B. Origin of the jugular sacs.....	25
C. Origin of the renal sacs.....	35
a. The retroperitoneal sac.....	35
b. The iliac sacs.....	36
c. The cisterna chyli	36
D. The thoracic duct.	40
2. Primary lymphatic sacs in human embryos.....	45
3. Primary lymphatic sacs in rabbit embryos.....	47
4. Primary lymphatic sacs in cat embryos.....	48
5. Primary lymphatic system in birds, posterior lymph heart and jugular lymph sac.....	50
6. Primary lymphatic system in amphibia, anterior and posterior lymph hearts.....	55
V. Peripheral lymphatic system	59
1. In the pig.....	59
A. From the jugular sacs.....	59
B. From the renal sacs.....	63
2. In other forms.....	65
VI. Origin and development of lymphatic glands and their relation to primary lymph sacs	65

	PAGE
VII. Comparative morphology of primary lymph sacs, lymph hearts, lymph glands and amphibian lymph sacs.....	70
VIII. Various other theories in regard to the origin and development of the lymphatic system.....	72
1. Mayer-Lewis Anlagen	72
2. Extra-intimal and perineural spaces and fenestration.....	75
3. Growth of lymphatics by the addition of tissue spaces.....	77
IX. Conclusions	82
X. Literature	83

111
S11
1913

THE ORIGIN AND DEVELOPMENT OF THE LYMPHATIC SYSTEM.¹

By FLORENCE R. SABIN.

(*From the Anatomical Laboratory of the Johns Hopkins University, Baltimore.*)

I. INTRODUCTION.

1. MORPHOLOGY OF THE VASCULAR SYSTEM, THE ANGIOBLAST.

Until the past few years our knowledge of the morphology of the lymphatic system was in a very unsatisfactory state. Our conceptions of such main questions as the origin of the first lymphatics, the time of their appearance, the relations of the lymph hearts and sacs of the amphibia to the lymph glands and ducts of higher forms, the relation of the lymphatics to serous cavities and to the various forms of tissue spaces, and the development of the lymphatic vessels within an organ have been so vaguely understood that the opinions of no two investigators approached agreement.

A clear conception of any system must be based on an understanding of its fundamental morphology, as, for example, the morphology of the arterial system was placed upon a satisfactory basis by the discovery and comparison of the aortic arches in different animals. An example more closely in touch with our knowledge of the lymphatic system is the study of the fundamental morphology of the blood vascular system. Our knowledge of this is in the process of being built up, but a foundation has been laid for the clear understanding of the vascular system in a series of discoveries. The first and most fundamental of these is that the blood vessels arise as blood islands in the extraembryonal membranes. The earlier embryologists on the other hand believed that the first vessels were spaces without walls, which the pressure of the circulating blood hollowed out. The best description of the

¹ Aided by a grant from the Baltimore Association for the Promotion of University Education of Women and by the Carnegie Institution of Washington.

origin of blood islands and the best analysis of the meaning of this discovery is to be found in two works of His, "Untersuchungen ueber die erste Anlage des Wirbeltierleibes" in 1868 (pp. 95-103) and in "Lecithoblast und Angioblast" in 1900 (pp. 268-295). The discovery of blood islands, however, dates back to the work of Wolff (154) and Pander (101), who introduced the name, and perhaps no one subject in embryology has a more extensive literature. Certainly a most interesting account of the development of the vascular problem can be followed through the pages of von Baer (7), Prévost and Lebert (108), Remak (124-125), Reichert (121-123), Koelliker (67-71) and Thoma (148). The second great advance was the discovery that blood vessels throughout are lined by endothelium (Hoyer 48), which followed soon after the corresponding discovery in lymphatics by von Recklinghausen. The third discovery involves the proof that blood vessels grow by the sprouting of their endothelium, Prévost and Lebert (108), His (47), Rouget (126) and Arnold (5); the fourth that the main vessels of the body wall, including the posterior part of the aorta (Evans 33-35), even the anterior part of the aorta and lateral heartanlagen, arise as a capillary plexus or as solid angioblast cords (Bremer 15), which invade the body from the extraembryonal membranes. Finally a complete conception of the development of the vascular system is based on the theory that the blood vessels of the extraembryonal membranes invade the body wall (His 47), and that within the body wall these capillaries of endothelium gradually invade or spread over the body so that there are definite vascular and non-vascular layers and zones. His (47) discovered the fact that blood vessels grow into the central nervous system; but the theory of vascular and non-vascular zones which is essential to an understanding of the development of the vascular system we owe to Mall (80-87). It has been worked out by his pupils, notably Evans (33-35). Its meaning can be grasped by studying figs. 4, 5 and 6 in connection with fig. 437 (Evans 35), which all show that the skin dorsal to the central nervous system is a non-vascular zone, which for a long time is not reached by the blood vessels in the centrifugal growth.

This series of discoveries, notwithstanding the gaps and uncertainties in our knowledge of the early stages which were well brought out by Minot (98, pp. 483-485) and by Evans (35, pp. 551-567) in 1911, uncertainties in part since removed by the work of

Bremer (15), offers a consistent as well as a constructive theory of the vascular system which goes far to modify the force of the following words of Koelliker quoted by Rückert and Mollier (128, p. 1019): "Ueber die erste Bildung der Gefäße und des Blutes herrschen wohl ebenso viele Ansichten, als Forscher sich ueber diesen Gegenstand ausgesprochen haben, was auf jeden Fall beweist, dass derselbe zu den schwierigsten gehört." The theory thus outlined is that the fundamental morphology of the vascular system is based on the specificity of the endothelium, or in the words of His (47, p. 325), on the fact "Zu den am frühesten sich sondernden Gewebsanlagen gehört der Gefäßskeim oder Angioblast. Seine Sonderung erfolgt sehr scharf, und sein Wachstum geht nach durchaus eigenthümlichen Gesetzen vor sich." It includes the discovery of Mall (83-87) that endothelium may produce reticulum, a process which he has shown takes place both in the liver and in the heart; but maintains that there is an early differentiation of two tissues, namely, endothelium and mesenchyme,* so that the angioblasts once formed give rise to all the vascular endothelium of the body.

The opposing theories in the varying forms of the origin of blood vessels from tissue spaces, their growth by the addition of tissue spaces or by the addition of connective tissue cells, and the differentiation of endothelium from mesenchyme over extensive areas within the body wall find their most recent evidence in the works of Rückert (127), Rückert and Mollier (128), Hahn (40), Bonnet (14) and Maximow, 1909 (90, p. 511). These views, however, must be traced through the earlier works of Götte (37) and Reichert (121-123). The evidence for the continued origin of blood vessels from the mesenchyme is for the most part from the interpretation of appearances in sections. Hahn's work, however, in connection with the difficult point of the origin of the heart and aorta is experimental. He removed the vascular membranes on one side of early chick embryos and obtained a heart anlage and aorta on both sides. He thought that he could entirely exclude a growth from one side to the other as well as remnants of the membranes on the same side and concluded that both heart and aorta arise in situ from the mesenchyme and not from the endoderm of the yolk sac not from an ingrowth from the extraembryonal membranes.

2. MORPHOLOGY OF THE LYMPHATIC SYSTEM.

It will be proved in the following pages that the lymphatic problem is closely connected with, or rather is a fundamental part of, the vascular problem, so that the study of the one throws light on the other, or, to put it more strongly, that the same kind of evidence is needed to solve both problems. The fundamental morphology of the lymphatic system has, however, been put on a more satisfactory basis than that of the vascular system, for it has been seen in the living embryo that the first lymphatics bud off from the veins. Moreover, lymphatics begin at a comparatively late stage, long after the formation of the blood islands has ceased.

II. HISTORICAL.

1. VASA SEROSA.

Until the primary origin of any system is known our conceptions of it are necessarily hazy and vague and this is nowhere better illustrated than in connection with the lymphatic system. The views of the eighteenth century may well be summed up in the hypothetical "vasa serosa" of Boerhave (13), Haller (41) and others, which were tiny channels too small to allow corpuscles to pass, supposed to connect arteries, veins and lymphatics at their tips. The conception which underlies vasa serosa may be traced back to the experiments of Nuck (100), who injected air into the arteries and found it returning in the lymphatics, as may be represented in the diagram of fig. 1. Vasa serosa meant the idea that the arteries finally branched into vessels too tiny to carry the corpuscles, but the term likewise represents a whole series of vague conceptions, such as Bichat's (12) absorbents and exhalants, which sought to make definite some idea of the nature of lymphatics.

2. LYMPHATICS WITH OPEN MOUTHS.

In the latter half of the eighteenth century the conception of definite "vasa serosa" was modified through the work of William Hunter (50) and Munro, who believed that lymphatics began with open mouths. The views of these English observers, as shown by Cruikshank (23), involved the idea that the mouths of the lymphatics opened directly onto the surface of the body, into the cavity of the intestine and the air sacs of the lungs, as well as into the connective tissues. The theory was also involved with discussions of the lymphatics as the exclusive

organs of absorption for the body, and the physiological theories of filtration.

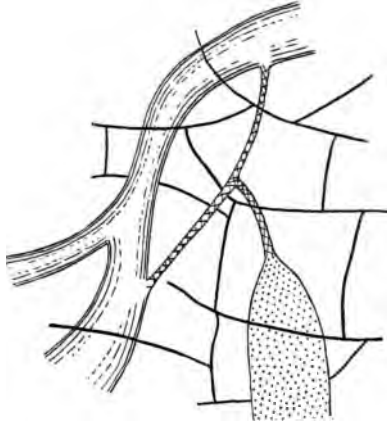


FIG. 1.—Diagram to show the “vast serosa” of Boerhave, Haller and others. The veins are striped, the lymphatics are dotted, and the “vasa serosa” which connect them are cross-hatched. The connective tissue is indicated by lines.

The doctrine, or as His (44) terms it, “the dogma of lymphatics with open mouths,” was first combated by a definite counter theory by Schwann (139), following his discovery of the cell in the animal body. The theory of Schwann and notably of Virchow (150) was that in place of vasa serosa and hypothetical connections which did not exist hollow connective tissue cells spanned the gaps between blood vessels and lymphatics, fig. 2.

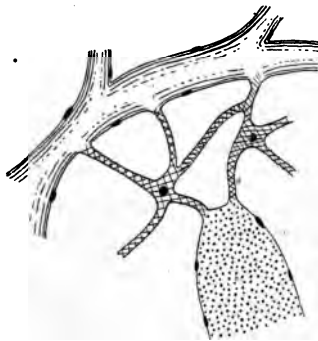


FIG. 2.—Diagram to show the theory of Schwann and of Virchow that the blood vessels are connected with lymphatics by hollow connective tissue cells. The veins are striped, the lymphatics dotted and the hypothetical lymph vessels are cross-hatched. The nuclei denote the fact that cells have been discovered.

3. DISCOVERY OF ENDOTHELIUM.

In contrast to these hypothetical relationships of lymphatics, the work of von Recklinghausen (119) gave the first definite theory which was actually based on the interpretation of direct observations of the lymphatic capillary. Through the use of silver nitrate solutions reduced in direct sunlight, he discovered that the lymphatics are lined by a definite cell layer of endothelium. This is clearly one of the most important discoveries in histology. Von Recklinghausen interpreted the silver pictures to mean that the finest lymphatic capillaries were lined by an epithelium, but that these capillaries communicated by wide openings with *Saftcanäle* or lymph radicles, which formed the real rootlets of the lymphatics. Subsequently he described more definite openings or stomata in the serosa of the diaphragm (120).

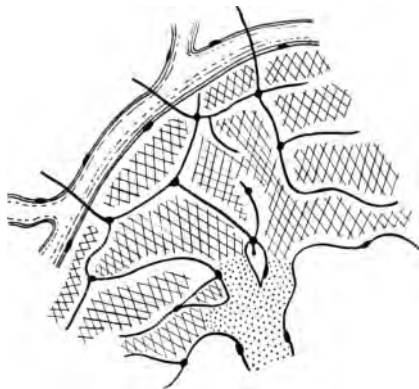


FIG. 3.—Diagram to show von Recklinghausen's theory of the relation of lymphatics to tissue spaces. All of the tissue spaces are cross-hatched to indicate hypothetical lymphatics.

The von Recklinghausen figures seemed to offer a perfectly satisfactory demonstration of the relations of open lymphatics, especially in view of the fact that the great majority of anatomists already believed in open lymphatics. But it is of great interest to follow the writings of His at this period. His (44), who was just publishing in favor of open lymphatics, and saw von Recklinghausen's paper as his own was going to press, at first recognized in von Recklinghausen's descriptions a confirmation of his own views. On careful studying of the silver pictures, however, His (45) saw that the discovery of endothelium was the very step which, instead of proving that lymphatics are open and

without definite limits, tended rather to throw the balance of proof in the opposite direction; that the discovery of endothelium gave the conception of lymphatic capillaries not as indefinite spaces without walls, but as limited by a definite membrane. To quote his words (45, pp. 457-458), "Wenn ich nach den eben gemachten Auseinandersetzungen der einen Aufstellung v. Recklinghausen's, nämlich derjenigen vom Vorkommen eines Epithels in den feineren Lymphgefäßen des Diaphragma vollkommen beistimme, so bin ich durchaus anderer Ansicht als jener Autor hinsichtlich der Saftcanälchen und ihrer Beziehung zu den Lymphgefäßen; fürs erste nämlich leugne ich das Vorkommen von Saftcanälchen in dem Sinn von v. Recklinghausen, und zweitens halte ich die Verbindung der von ihm als Saftcanälchen gedeuteten Theile mit den Lymphgefäßen nur für eine scheinbare," and later in his interpretation of the silver picture he says: "Je dünner und blasser die supervasculäre Schicht ist, um so leichter wird es den Anschein haben, als ob die hellen Figuren der intervasculären Bindegewebskörper unmittelbar in die Lymphgefäße selbst einmündeten, um so eher wird man uebersehen, dass in Wirklichkeit der Zusammenhang jener Figuren nicht mit den Gefäßen, sondern mit den Gefäßen ueberlagerten verzweigten Körpern stattfindet." Further he adds that if the openings pictured by von Recklinghausen exist, there would be no reason why any injection of a finely divided mass should not fill practically all of the *Saftcanälchen* or tissue spaces as expressed in fig. 3, and this is obviously not the case. Thus after a careful study and interpretation of the silver reaction on the lymphatic vessels, on the albuminous fluid of the interspaces, and on the cell bodies of the connective tissue corpuscles, His was led to believe that the significance of the von Recklinghausen discovery was that lymphatics are lined throughout by an endothelium rather than that the silver method demonstrates open lymphatics.

III. EMBRYOLOGY.

1. INTRODUCTION.

It is, I think, clear that the problem in this state could only be attacked successfully by embryology with the purpose of establishing the fundamental morphology of the system. The importance of this is best expressed by His (44, p. 223), "Von den mancherlei offenen Fragen, die seit mehr denn 200 Jahren in den anatomischen Schriften

ueber das Lymphsystem discutirt zu werden pflegen, bietet wohl keine ein so unmittelbares physiologisches Interesse dar, als gerade die Frage nach dem Ursprung der Lymphgefäße in den Körperorganen. Mag man sich ueber die Bildung der Lymphe und ueber die bei ihrer Fortbewegung wirksamen Kräfte eine Theorie machen, welche man will, so fordert diese als ganz unerlässliche Grundlage eine praezisere Vorstellung von dem anatomischen Verhalten der ersten Wurzeln des Systems, sei nun eine solche Vorstellung wirklich aus der Beobachtung entsprossen, sei sie nur hypothetisch angenommen." It is without question that a comprehension of the relations of the lymphatic capillaries depends on an understanding of the fundamental morphology of the system.

2. LYMPHATICS AS DILATED TISSUE SPACES.

The relation of the lymphatics to tissue spaces formulated by von Recklinghausen was entirely in agreement with the early crude investigations of foetal conditions recorded by Breschet (16). It was the practically universally accepted idea up to 1900 that lymphatics arose by the dilatation of tissue spaces caused by the fluid that exuded from the blood vessels; that this dilatation of the spaces began in the periphery, and that the vessels gradually approached the veins and joined them. Thus the growth of the lymphatic vessels was thought to be from the periphery to the center. This view was well stated in 1894 by Gulland (39), who found the lymphatics first in the subcutaneous tissue and then along the extremities in fetuses, human, bovine and rabbit, between 3 and 4 cm. long, and thought that these vessels subsequently joined the veins. He thought that the force of the fluid in the tissues formed the lymphatics, so that they arose by an entirely different method from his conception of the origin of blood vessels (p. 467).

3. BUDGE'S WORK.

An account of the newer work on the origin of the lymphatic system must begin with Budge (17-22). Budge was led to a study of the lymphatic system in birds through noticing the great variations of the posterior lymph hearts in the adult (21). The posterior lymph hearts had been seen in birds by Panizza (103), A. F. J. Mayer (quoted by Stannius) and Stannius (142). Budge had already begun

to study lymphatics in the adult liver (17) and in bone (18) by means of injection, and in 1880 (19) he described studies of a system of canals which he could inject in mesoderm of three-day chick embryos. These injections, which can be easily repeated, giving the exact patterns of Budge, were really made into the extraembryonic coelom, as has been shown by Mall (81) and myself (129), and have nothing to do with the lymphatics. They are not, as Budge himself noted, lined by endothelium; indeed Budge himself was most cautious in his interpretation, for he says (19, p. 325), "Mit Absicht habe ich den Ausdruck Lymphgefäßsystem für das eben beschriebene Canalsystem vermieden, weil mir hierfür noch nicht Anhaltspunkte genug gewonnen zu sein scheinen. Und doch liegt der Gedanke hieran nahe." In 1881 Budge (20) announced the important discovery that there was a plexus of lymphatic vessels accompanying the allantoic arteries of chicks easily injected from the sixteenth to the eighteenth day of incubation, and that these injections ran to the thoracic duct. The next year (21) in a fuller paper he described that these allantoic lymphatics not only entered the thoracic duct but drained into the posterior lymph heart, which played a great rôle in relation to the allantois. He injected the lymph heart through the allantoic vessels by the tenth day, and noted that the posterior lymph heart was preceded, as seen in the living chick (8 to 12 days), by veins, and that subsequently the lymph heart appeared, which could be distinguished by the fact that it pulsed with a different rhythm from the blood vessels. After Budge's death his work was brought together by His (22) and formulated somewhat in this manner: There are two lymphatic systems, a transitory and a permanent system, the first consisting of spaces in the extraembryonal membranes analogous to the coelom and found only in early stages. The second, consisting of the true endothelial-lined vessels which accompany blood vessels—which Budge so successfully injected. The thoracic duct he thought arose from the first system and was the only permanent part of the first system. Thus Budge's work, which was a reaching out into a dark field and had genuine important discoveries, was in line with the prevailing theory that lymphatics are related to tissue spaces, and introduced a misleading conception that lymphatics form after the same manner as the coelom and indeed in part as an extension of the coelom as far as the primitiv system and thoracic duct are concerned.

4. STATEMENT OF THE ARGUMENTS FOR THE VENOUS ORIGIN OF THE LYMPHATIC SYSTEM.

I shall now take up the proof of the theory that the lymphatic system is derived from the venous system, and will subsequently analyze the opposing theory, which, in its most recent form, is that lymphatics grow on the addition of tissue spaces (Kampmeier 66 and 66a).

The essential elements in the establishing of the venous origin of the lymphatics have been (1) the proof that lymphatic capillaries grow by the sprouting of their endothelial wall and not by the addition or hollowing out of connective tissue spaces. This argument is known as the method of growth by sprouting. (2) The proof that lymphatics gradually invade the body from center to periphery, establishing the point of lymphatic and non-lymphatic zones. This means that the main ducts grow as capillaries by the same method as the peripheral capillaries. This argument is known as the one of lymphatic and non-lymphatic zones. (3) That the original lymphatic ducts bud off directly from the veins. This is known as the origin of the lymph sacs by budding.

Thus the study involves three elements: The nature of the lymphatic capillary, the nature of the lymphatic ducts, and the nature of the first lymphatics or the lymph sacs. Each one of these three lines of proof involves the study of endothelium; the first lymphatics are buds from the endothelial lining of the veins; these endothelial buds invade the body down to the ultimate capillaries by the cell division of their endothelial wall.

A. GROWTH OF LYMPHATIC CAPILLARIES BY SPROUTING.

A study of the method of growth of lymphatic capillaries is necessarily associated with the study of the method of growth of blood capillaries, for as far back as the time of Schwann (139), who first saw the capillaries in the living tadpole's tail and who vaguely distinguished those which carried blood from those which had no corpuscles, there is a clear recognition that the two forms of capillaries grow by the same method. This method Schwann thought to be by the addition of hollow connective tissue cells.

The term growth by sprouting was introduced quite recently by His, but the first description of the process dates back to the year 1844. to

the work of Platner (106) and of Prévost and Lebert (108). Platner described the growth of capillaries as seen in the living tadpole's tail. He analyzes Schwann's idea of the addition of cells as necessarily merely an inference from the appearance of the vessels in one stage, for when actually seen growing new cells are never added on. He says that rather each new vessel is a process of a preceding one and describes these processes as long, thin *Ausläufer*, which form loops and soon show a double contour. He says that the tiny processes never contain nuclei and are not cells, so that the interpretation of Schwann of the addition of cells could not possibly hold. Platner says that the same process of growth in the living tadpole had been observed by Prévost and Lebert. Indeed, in the same year (108) they published a series of four papers, in which they describe the growth of capillaries, not only in Batrachians, but more in detail in connection with the blood islands in the chick. They definitely use the term centrifugal growth and describe the process in the following graphic terms (p. 239): "Les vaisseaux poussent des saillies latérales par décollement partiel des lamelles du feuillet vasculaire, saillies plus ou moins arrondies ou pointues, allongées, formant des espèces d'éperons qui souvent finissent par se rencontrer, provenant de deux côtés différents, et établissent ainsi des vaisseaux de communication." They thought that the processes or "spurs" were for the most part hollow from the start and state that the larger vessels grew by the same method as the smaller.

In 1846 Koelliker published two papers (67 and 68), in one of which he announced the discovery of lymphatic capillaries in the tadpole's tail (67). He described the characteristic differences as seen in the living form between the two types of capillaries, the irregularities and more numerous processes of the lymphatics as well as their connection with the superior and inferior caudal lymph trunks. In the other paper he gives a most valuable analysis of the views of the early embryologists, Wolff, von Baer, Reichert and others on the problem of the growth of vessels. He takes up the various views of growth (p. 118) which were then being discussed, namely, growth (1) by the addition of unbranched cells, or (2) by branched or stellate cells, or (3) by spaces hollowed out by the force of the heart beat, or (4) from spaces produced by fluids in the tissues as well as (5) by the then new work of Platner, Prévost and Lebert, showing centrifugal

growth, and concludes that the vascular and lymphatic capillaries grow by the addition of branched or star-shaped cells.

Four years later, 1850, Remak (125) rediscovered the lymphatics in the tadpole's tail by watching the living embryo, and, without knowing of the preceding work, he hurriedly published an even more graphic description of the form and growth of lymphatics than Koelliker's. Subsequently, after seeing Koelliker's work, he added a note (p. 182-183) to emphasize the fact that if the growth be watched in the living form star-shaped cells are never added—neither to the blood capillaries, nor to the lymphatic capillaries, nor, he adds, to the growing nerves. Many years later, 1886, Koelliker (71, p. 34), in an article mainly on nerves, corrected his view of the growth of the lymphatics by the addition of connective cells in favor of the theory of growth by sprouting.

The years from 1863 to 1867 represent a great advance in our conceptions of the lymphatic system. The historical article of His (44) on the lymphatic system must form the basis of any work which deals with the development of the problem. In 1865 Stricker (143) published a paper in which he expresses a doubt in regard to Koelliker's discovery of lymphatics in the tadpole's tail, on account of the fact that undoubted blood capillaries had also the blind sprouts. He, however, submitted the blood capillaries to an experimental study of great interest. He stimulated them with the vapor of ammonia and gives the following description of his results:

Ich sah ein Gefässstück, welches seinem Baue nach unzweifelhaft zu den Kapillaren gezählt werden musste, sich im Laufe von fünfzehn Minuten zweimal bis nahe zum Verschwinden des Lumens verengern und bis zum Breitendurchmesser eines Blutkörperchens wieder erweitern. Ich sah varicöse Gefässe sich so verändern, dass der Bauch an Stelle des Halses zu liegen kam, und umgekehrt. Mit den Formveränderungen ging gewöhnlich noch eine andere Veränderung Hand in Hand. So wurde beispielweise eine eingeschnürte Kapillargefässstrecke so blass, wurden deren Conturen so undeutlich, dass ich sie kaum mehr von der Umgebung unterscheiden konnte, während nach bis zwanzig Minuten das Gefäss wieder in seiner ursprünglichen Deutlichkeit zu sehen war.

Thus he discovered the contractility of endothelium. He found that electricity was a still better stimulant for observing this contractility. He discusses his results from the standpoint of determining the nature of the capillary wall, especially in connection with the silver preparations of Aeby, Auerbach and Eberth (quoted from Stricker 143),

which has already given the suggestion of a lining of flattened cells for blood capillaries. Stricker saw clearly that the wall of the capillary was of living protoplasm, but was confused in regard to whether the blood stream was inter- or intra-cellular. He was perplexed also in regard to the existence of cells or not, since he was dealing with the growing tip, which we now know to be a syncytium. This idea occurred to Stricker, as is plain in these words, "Dass bei dem Aufbau von Kapillargefässen ueberhaupt Zellen mit einander verschmelzen, und dass solche Verschmelzungsspuren später einmal durch Silber-niederschläge kenntlich werden können, lässt sich ferner auch nicht leugnen." Stricker also noted the sprouts of blood vessels as indicating their method of growth.

I have already taken up the fact that von Recklinghausen's work on the endothelium of lymphatic vessels, studied by means of the silver nitrate method, settled the question of the cellular nature of the lining of lymphatics, especially in connection with the analysis of the work by His, who had regarded lymphatics as vessels without walls (44, p. 229), and saw by a thorough study of preparations made by the silver method that lymphatics were lined throughout by endothelium.

In 1867 Langer (75), as a conclusion to a series of studies on the lymphatic system in amphibia, published a study of the lymphatics in the tadpole's tail. He was able to make complete injections of the blood vessels and the lymphatics and gives a most interesting analysis of specimens as observed in alcohol from the standpoint of weighing the evidence on the method of growth. He describes the uninjected capillary as often appearing to have a single contour, while the injected one had the complete wall. He could not find in the alcoholic specimen the tiny processes previously described in the living form. He noted, however, the larger sprouts, the loops and blind processes, and says that the nuclei are definitely within the contour of the wall of the capillary, so that he makes the distinction between the vessel and the surrounding tissue. He concludes that the various forms of the processes must indicate the process of centrifugal growth for the lymphatic capillary as well as for the blood capillary.

Again in 1873 the growth of the lymphatics and blood vessels by the method of sprouting was observed in the tadpole's tail and very clearly described by Rouget. By the use of curare he was able to keep the larva still. He also emphasizes the great number of tiny filaments

on the lymphatics and the fact that they never join with the connective tissue cells.

Thus the observation of the growth of capillaries by sprouting was made over and over again where there was the chance of seeing the process in the living form or of obtaining injected specimens, and yet during all these years the observations were not generally accepted, since as the fundamental morphology of the lymphatic system was unknown, the significance of the observations could not be understood.

It is, however, not true that every observer who studied the living tadpole's tail described the process of the sprouting of the endothelial wall of blood vessels and of lymphatics as showing the method of growth. Indeed at least three observers have doubted whether there are two different kinds of capillaries in the tadpole's tail. I have already noted that Stricker doubted the presence of lymphatic capillaries in the tadpole's tail.

Wysotzky (155), whose work, since it is published in Russian, I quote from Mayer (89), thought that the capillaries without blood were merely young blood capillaries.

Sigmund Mayer (89), in an interesting and valuable paper, "Ueber die Blutleerengefäße im Schwanz der Batrachierlarven," gives first an excellent analysis of the literature on the subject of the capillaries in the tadpole's tail. He brings out the fact that it was the presence of an occasional red corpuscle in the "lymphatic" which was the stumbling block in accepting the presence of lymphatic capillaries. He then records an observation which in reality cleared up the entire difficulty in regard to the "empty capillaries." As E. R. Clark (26) has shown, Mayer, however, failed to see the bearing of his own observations. Mayer used curare and the electric current to anaesthetize the larva and then covered the tail with a cover slip. When he sucked the water out from under the cover slip he noted that the blood would gradually stop flowing or even go in the reverse direction in the blood capillaries and that the vessel would collapse, so that it looked like a solid cord. Thus he also observed the contraction of endothelium. Furthermore, he noted that the "other empty vessels" (namely, lymphatics), contracted more frequently than the blood capillaries. When, however, he studied these other empty vessels he thought that the occasional cells in them flowed now toward the center, now toward the periphery, and he ended with the somewhat vague

and erroneous conclusion that the empty vessels, though they might have some of the functions of the lymphatics of the adult, could not properly be grouped with them. We now know that the two sorts of empty vessels are (1) the collapsed blood vessels which Mayer himself observed and (2) the true lymphatics (E. R. Clark, 26).

The work of Mayer, however, has another point of great interest to us beside the important observation of the contractility of endothelium, namely, what we call Mayer-Lewis anlagen. Mayer noted the long strands of cells (endothelium) which often span the gap between two capillaries; indeed, he figures them, some that look entirely solid and some that have, here and there, a patent lumen. In reality both blood capillaries and lymphatic capillaries have these strands and sprouts, and it is one of the most remarkable qualities of the two forms of endothelium that no sprout from a lymphatic capillary ever joins a blood capillary and vice versa. However, Mayer's mistaken idea that these strands of cells connected with two different kinds of capillaries, namely, the vessels which are always empty, in other words, the lymphatics and the blood capillaries, aided in his confusion of the lymphatics with the blood vessels. Besides these strands of endothelium he saw small hollow vessels, having the same lining as the capillaries, which he thought to be entirely isolated. These he interpreted as follows (p. 236):

Unseren Erfahrungen nach sind nun die beschriebenen Bilder nur gewisse Stadien der Ausschaltung von Bestandtheilen des Blutgefäßsystems aus der normalen Circulation. . . . Bei genauerem Studium der letzteren Formationen ergibt sich, dass die Wandungen derartiger mit freien Spitzen in das Gewebe hineinragenden, und ausserhalb jeder Kommunikation mit Gefäßröhren stehenden Fragmente bezüglich des Aufbaues ihrer Wand entweder mehr den Typus der blutleeren oder demjenigen der bluthaltigen Röhren sich nähern.

Most of the apparently isolated anlagen seen in sections are due to the collapsing of the vessels which connect them with the rest of the capillaries through the contractility of the endothelium which Stricker and Mayer observed. In sections they may occur in great numbers through the contraction of the endothelium due to the fixing agent. The possibility of entirely isolated vessels and the meaning of the apparently isolated vessels are fully discussed later in connection with the theory of the growth of lymphatic capillaries by the addition of Mayer-Lewis anlagen in section VIII.

If it be established that the peripheral capillaries grow entirely from preceding capillaries, then it is a perfectly logical hypothesis that the lymphatics grow from the veins, from center to periphery, rather than from the periphery to the veins. This hypothesis was advanced by Ranvier (113), based on the evidence of the method of growth of the lymphatic capillaries within the peripheral plexus. Ranvier (110-118), in a series of studies between the years 1895 and 1897, made injections of lymphatics in a variety of embryos. For example, he injected the exceedingly abundant plexus in the mesentery of a foetal pig 10 cm. long, as well as in the rat and rabbit. He also injected the lymphatics in the amphibia. These injections gave him an extensive knowledge of the form of the developing lymphatic plexuses, the valves looking like collarettes in the walls of lymphatic ducts, the irregular capillaries with blunt terminal knobs, of which especially large ones are often found in the mesentery as well as the slender sprouts, either ending free or as bridging a gap in the network to another vessel. From all of these forms of growing lymphatics Ranvier saw that the growth of lymphatics was from center to periphery, and even though he thought that the lymphatics did not begin in a mammalian embryo until it was about 10 cm. long, a stage at which the embryology of the system may be called complete, nevertheless he went a step farther than all of the preceding observers, for he deduced the hypothesis that the lymphatics grow from the veins. He likened their growth to the growth of a gland (113). In my own work I was led to the same view by tracing the lymphatics in younger and younger embryos back to the veins, and the next section will include the evidence which I regard as essential to establish the hypothesis of Ranvier as the correct theory of the origin of the lymphatic system. The account of the evidence for growth by sprouting is, however, not yet complete. As far as the evidence can be obtained by the observation of injected specimens, it was especially well described and figured by MacCallum (79). He added the observation of watching the injection under the microscope and showed that extravasations are due to explosive rupture of the lymphatic wall.

Injected specimens of developing lymphatic capillaries are now well known; excellent figures are given by Bartels (10, figs. 9 and 10, p. 43). They show a large number of sprouts, because they are taken from the skin of a pig embryo 6.5 cm. long. This is the exact stage when the secondary, superficial, fine-meshed plexus is just growing

from the deeper coarse-meshed or subcutaneous plexus, and, since the secondary plexus is growing actively, it shows many sprouts.

The proof of growth by sprouting from injections is always an interpretation of appearances, and though it is a logical inference, one must turn to the observations of the living form for conclusive proof. This has been given by E. R. Clark (25-26), who has again restudied the classical object, the tadpole's tail. The introduction of chloreton anæsthesia is one of the factors that have made this study possible, since the same specimen can be kept alive for weeks, notwithstanding repeated doses. Dr. Clark's device of an upright chamber, so arranged that the tadpole can be kept upright without the pressure of the cover slip, and the finding of larva of the form *Hyla pickerlingii* in the spring of 1910, a form which has few pigment cells, have all combined to make his studies so valuable and convincing. He has described not only the complete history of a lymphatic capillary, watched through periods of weeks, but has followed every connective tissue cell in the neighborhood (30) through several generations. Dr. Clark has shown that the wall of the lymphatic capillary is in ceaseless activity. In the living form the wall is in part hyaline, in part granular. The nuclei are surrounded and obscured by granular protoplasm, so that they are clearly seen only when dividing. The wall is of irregular thickness, often extremely delicate, and from the sides and tips are sent out numerous tiny processes, some hyaline and some granular, which may be well described as amœboid. A few of these persist and grow into permanent lymphatics. Most of them are withdrawn. These tiny sprouts, indicating the functional activity of the vessel, do not disappear in alcoholic specimens (26, p. 403). They are at first without nuclei, but nuclei wander into them from the parent stem; indeed, two nuclei may pass each other as they advance or recede. This proves that the growing amœboid wall of the lymphatic capillary is a syncytium and explains the failure to obtain the silver markings at the growing tip. The silver markings indicate the more stable lymphatic capillary.

E. R. Clark's most recent observations (30) show that increased activity of the lymphatic wall in the sending out of many of the tiny processes is a sign of growth, so that an area can thus be selected to watch the processes of the formation of new vessels.

As far as the relation to the surrounding connective cells is concerned, the growing, and, we might also say, the functioning, amœboid tips avoid the cell bodies and processes of the mesenchyme cells.

Thus there can be no question in regard to the method of growth of the peripheral capillaries. Both blood and lymphatic capillaries grow by the activity of the protoplasm of their walls. The endothelium at the growing tip is a syncytium, the processes advance and recede; they are tiny, hair-like vessels, and they receive their nuclei from the parent stem. There are differences in the appearance of the two types of capillaries probably associated with the presence and absence of the circulating blood and the difference of function. The lymphatics have very many more tiny sprouts, only a few of which are permanent; that is, their wall is much more amœboid and the vessels much more irregular. Thus on positive evidence, namely, the observation of the process in the living form, which is the best evidence known to anatomical science, we are justified in the conclusion that capillaries grow by sprouting.

B. LYMPHATIC AND NON-LYMPHATIC ZONES.

The second argument in favor of the venous origin and the centrifugal growth of lymphatics by their own endothelium is that it can be shown that lymphatic vessels gradually invade the body from certain limited centers. Thus if injections of vessels are made from these centers in larger and larger embryos an ever-increasing zone of lymphatic capillaries can be demonstrated. Moreover, for each stage the zone of vessels is constant.

I began my work with the study of the development of lymphatic glands in the embryo pig and to obtain injections of them introduced the needle into the foot pads. By taking younger embryos a stage was soon reached when no injections of the foot pads ever entered lymphatics, but at these stages one could still inject lymphatics in the skin over the body. This was the beginning of the proof that lymphatics bud off from the veins and grow peripheralward instead of the reverse (Sabin 129).

In an embryo pig 5 cm. long (Sabin 130, fig. 5) a simple plexus of lymphatic capillaries has almost entirely covered the body. I say capillaries advisedly, adopting Ranvier's (118, p. 74) criterion of valves for lymphatic ducts in contrast to their absence in the capillaries. The body is first invaded by lymphatic capillaries, and the primary plexus shown in fig. 5 (Sabin 130), which is the anlage of the deep, subcutaneous plexus, was injected by a single puncture of the needle.

Subsequently this plexus develops valves, but at this stage the entire lymphatic system can theoretically be injected from any one vessel, because there are no valves, or, in other words, because it is entirely a capillary system. Nearly the same stage is shown for a human embryo in figs. 505 and 506 (Sabin 134). This was a remarkable specimen, in which air filled the lymphatics, and it has the additional interest of showing the beginning of valves in the vessels of the skin, namely, those of an occipital duct and in the axillary and inguinal ducts.

If earlier stages of embryo pigs are taken the cutaneous vessels, as shown by injecting them, gradually recede, as seen in figs. 1 to 4 (Sabin 130), to two points, one in the neck and one in the groin. The earliest lymphatics reach the skin in the neck of an embryo pig about 18 mm. long. All injections of the lymphatics of the skin of the anterior part of the body between the stages of 2 and 5 cm. long can be seen to run into two symmetrical sacs in the neck close to the jugular vein. These anterior lymph sacs, which have now been identified in a very great number of forms, can be found in pig embryos in earlier stages than the first cutaneous lymphatics. In my first paper I traced them back to the stage of 14 mm., when they are small empty sacs, close against the anterior cardinal vein. In reality they begin in the pig of 10 to 11 mm. and their method of origin will be discussed in the next section.

The argument of lymphatic and non-lymphatic zones thus consists in the fact that the first lymphatics are sacs close to the veins, and from this stage onward lymphatics can be injected in wider and wider zones until they cover the entire surface of the body.

It is necessary to prove that each zone represents the limit of the growth of the lymphatics for a given stage. To prove an injection is complete for any stage such as one shown in fig. 6 (Sabin 135), it is essential to have abundant material and to show by injection that the lymphatic tips always rupture along a given line when the pressure is increased. The shape of the lymphatic vessels at the line of growth will vary according as the vessels are progressing rapidly or not; for example, the rounded ends of fig. 2 (Sabin 129) are from an area where lymphatics grow slowly, while the margins of fig. 6 (Sabin 135), with numerous pointed sprouts, are from a rapidly growing zone. Furthermore, sections of the skin in the lymphatic and non-lymphatic zones show a sharp contrast in the presence or absence of the large lymphatic

capillaries, fig. 6 (Sabin 130), which are easy to identify. The method of sections is, however, inadequate to define the limits of growth, except for the large vessels, for the tiny lymphatic sprouts can only be found in sections when the plane of cutting is favorable.

This progression of the lymphatics in the skin has been confirmed by Polinski (107) in bovine embryos. The patterns which he shows make a most interesting comparison with those of pig, cat and human embryos. In the bird the same progression of zones is shown to some extent in the injections of Mierzejewski (96).

Beside the definite zones the lymphatics grow into definite layers. Ranvier (118) noted that the superficial lymphatics are deeper than the blood vessels. They follow the vessels into the skin and each plexus is deeper than the corresponding blood vascular plexus (Sabin 130). The development of the lymphatics into the intestine, as worked out by Heuer (43), illustrates the point of the definite layers into which the lymphatics grow particularly well. The lymphatics for the intestine grow from the retroperitoneal sac, which arises from the renal veins (Baetjer, 8). The vessels form a rich plexus in the mesentery and from this plexus a series of vessels grow into the intestinal wall and penetrate to the submucosa. Here they form at first a series of units shown by Heuer in fig. 10 (43), which has been copied as fig. 508 (Sabin 134). These primary vessels soon form the submucosal plexus of ducts, from which the mucosal capillary plexus and lacteals develop on the one hand and the serosal vessels on the other.

This fact, that the body is invaded by lymphatics, has an especial interest, because it was the clue which enabled us to trace back the systems of lymphatic ducts to their beginnings and show that they arise from the veins and grow toward the periphery. It is, furthermore, the key by which the development of the lymphatic system within each organ may be traced to the ultimate capillaries. It is considered again in relation to the development of lymph sacs, for there it can be taken up in connection with its exact relations to vascular and non-vascular zones. Both the blood vascular and the lymphatic systems invade the body by the growth of their capillaries. Each system has its definite zones and layers which can be followed in orderly progression. The invasion of the body by the blood capillaries is more complete than by the lymphatic capillaries; for example, the lymphatic capillaries do not grow into the central nervous system, nor into the interspaces between muscle fibers.

C. DIRECT BUDDING OF THE LYMPHATICS FROM THE VEINS.

As has been stated, it was the study of the decreasing zones of lymphatic capillaries which led to the discovery that the first lymphatics are sacs close to the veins. In mammalian forms the first lymphatics are the anterior lymph sacs which bud off from the anterior cardinal veins. These jugular lymph sacs were noted by Saxer (138) in 1896 in his extensive study on lymph glands, but he did not recognize their significance. They can readily be made out in sections of pig embryos 14-20 mm. long, or in sections of any mammalian embryo of the corresponding stages. The fact that they are the first lymphatics in mammals and that they lie close to the vein, separated only by the double endothelial wall, led me to the conclusion that the lymphatics budded directly off from the veins and it is gratifying to find that this reasoning has been justified by further investigation (Sabin 129, E. R. & E. L. Clark 29 and Hoyer 49a).

To follow the development of our knowledge in historical sequence the next step was taken by F. T. Lewis (76) in a series of excellent reconstructions of the anterior lymph sacs in rabbit embryos, the best figures we have had up to the present time of the form of the early sac. Lewis carried the study still further back and showed that the definite sac was preceded by a blood-filled capillary plexus. This blood-filled plexus has proved to be a very important observation. From the presence of blood Lewis made the logical inference, with the evidence at his command, that these vessels were blood capillaries and that hence lymphatics were transformed blood capillaries. The observation of the blood-filled vessels has been confirmed by Huntington and McClure (54) on the cat and by myself in human and pig embryos (132-133) and we have all accepted the theory of transformed capillaries until new evidence has now shown us the correct explanation of the presence of the blood.

This new evidence has been furnished by the work of E. R. and E. L. Clark (27-29). E. L. Clark began with the study of the allantoic lymphatic vessels in chick embryos. From the allantoic vessels she was led back to the posterior lymph hearts and the lymphatics of the body wall. The injections of Mierzejewski (96) show that the primary line of growth for the lymphatic vessels in the skin of the chick is along the thoraco-epigastric vein (96, figs. 1 to 4). E. L. Clark noted that in early stages the lymphatic vessels along this line

were filled with blood and that the blood in them was stagnant, in striking contrast to the rapidly circulating venous blood. The fact that the lymphatics invade the skin in the chick while they are in the blood-filled stage gives at once an advantage. By means of fine glass cannulas E. L. Clark was able to inject individual vessels of this blood-filled plexus in the living chick and watch the ink flow to the pulsating lymph heart and thence to the veins. These blood-filled vessels have the usual characteristics of lymphatic capillaries, in that they are larger and more irregular than blood capillaries, but the essential point in E. L. Clark's work is that by using a form in which the lymphatics could be seen in the living embryos she discovered a new criterion for recognizing lymphatics, namely, the stagnant blood in contrast to the circulating blood.

With this important criterion she has followed back the origin of the posterior lymph hearts. Since Sala (137) it has been known that in the chick the posterior lymph hearts develop opposite the lateral branches of the first five coccygeal veins (137, p. 269). Sala described the process as beginning during the sixth day by a hollowing out of spaces in the mesenchyme, but by watching the living chick embryo during the fifth day E. R. and E. L. Clark have seen that the skin over the first five coccygeal veins is a comparatively non-vascular zone, so that brilliant direct illumination enables them to see in the depth a series of tiny blood-filled buds close to the main coccygeal vein and its branches. The blood in these buds is of slightly different color from the circulating blood. They proved that these buds are always in connection with the parent vein, for a direct injection of them always runs over into the vein, but no injection of the peripheral blood capillaries ever fills them. Hence they bud off from the veins and are filled with blood from a back-flow from the parent vein. It is of course evident that this process cannot be seen in the body wall of the chick with the clearness with which every cell division can be followed in the tadpole's tail; nevertheless, the stagnant blood has been proved by injection to practically fill the developing vessels, so that it is an adequate criterion of the extent of their development.

By selecting a chick which shows these primary lymphatic buds and keeping it under observation under high power in a warm chamber E. R. and E. L. Clark (29) have been able to watch these blood-filled lymphatic buds join with each other to form a deep circumscribed lymphatic plexus, the anlage of the posterior lymph heart. It is clear

from Sala's description that it was this plexus which he described as the beginning of the lymphatics. While the deep lymphatic capillary plexus is being transformed into a lymph sac and pulsating lymph heart, lymphatic vessels are already growing toward the skin, where they gradually extend. They grow first along the lateral line to anastomose with the jugular lymphatics. There is a continuous peripheral growth of the lymphatics from the time of the first buds on, and the entire blood-filled stage takes but a few hours. Nevertheless, it is long enough to enable one to watch the buds form a plexus; the plexus becomes a sac and lymph heart, and the lymphatics from it spread out in the skin. Injections prove that in every stage of their development, from the primary buds onward, the lymphatics connect with the parent veins; thus there is no separation from the vein and subsequent rejoining, as all of the recent workers, Lewis, Huntington and McClure and myself, have thought from the difficulty of finding the connections with the veins in serial sections.

The posterior lymph heart in the chick is especially advantageous for study, since it is in the first place sufficiently superficial to be seen in the living embryo, and in the second place it is covered by a non-vascular zone. In mammalian embryos no caudal lymph hearts develop. It is very interesting to note in injected embryo pigs that the corresponding area in the skin at the root of the tail, instead of being a non-vascular zone, is an area of especially wide blood capillaries. This area, seen in fig. 467 (Evans 35), is constant, and, as it almost always retains a little blood, can be injected when a partial injection of the veins of the lower part of the body is desired.

The work of the Clarks adds the final proof of the theory that the lymphatics bud off from the veins. Moreover their method is so simple that anyone may convince himself of the essential results by a few hours observations. Their injections have corrected two errors, namely, the idea that the lymphatics are transformed blood capillaries and that they temporarily separate from the veins.

Thus I regard as proved the three points which have been stated as essential in establishing the origin of the lymphatics from the veins. Stated in reverse order they are: First, the lymphatic system begins as a series of endothelial buds which sprout out directly from the veins as a new type of vessels, namely, lymphatics. These buds unite to form plexuses which develop into sacs. These sacs may become lymph hearts. Second, from these lymph sacs or hearts lymphatic

capillaries gradually invade the body in orderly sequence in definite and characteristic zones and layers. The growth is always in the capillary bed, that is, all lymphatics develop as capillaries, and the earliest ones to develop become the first lymph trunks or ducts. Third, the method of growth throughout is by the sprouting of the protoplasm and the nuclear division of the endothelial of the capillary wall. The ultimate capillaries are distinguished by the absence of valves. In a word, there is a continuous growth and invasion of the body by lymphatic capillaries from the primary sprouts which bud off from the veins to the ultimate peripheral lymphatic capillaries.

IV. PRIMARY LYMPHATIC SYSTEM IN DIFFERENT FORMS.

1. PRIMARY LYMPHATIC SACS IN THE PIG.

A. GENERAL SUMMARY OF THE SACS.

I shall begin the section on the special anatomy of the lymph sacs by a detailed description of the origin of the lymphatics in the pig, since that is the best known form. I can now describe the origin of all of the lymphatic sacs in the pig and give the relation of the main ducts to all of these primitive sacs. On the basis of our knowledge of the lymphatic system in this form I shall give what is known of the lymphatic system in other mammals, in birds, reptiles and amphibia, and shall then be in a position to compare the primary lymph sacs in mammals with the amphibian lymph hearts and sacs.

In the pig the lymphatics bud off from the veins in two places, from the anterior cardinal veins and from the veins of the Wolffian body. There are two sets of paired sacs, the jugular and the iliac; and two unpaired sacs, the retroperitoneal and the cisterna chyli. In the most general terms the jugular sacs drain the anterior half of the body; the iliac sacs drain the posterior half of the body, while the retroperitoneal or præ-aortic sac drains the viscera. The cisterna chyli with the thoracic duct connects the jugular and renal lymphatics. In more specific terms the jugular lymphatics bud off from the anterior cardinal veins, form large sacs in the neck, from which lymphatics grow to the head and neck, the foreleg and thorax, and to the heart and lungs. From the left sac arises the jugular stem of the thoracic duct. All the rest of the lymphatic sacs bud off from the vena cava, where it is a part of the median mesonephritic vein, from the median mesone-

phritic vein and the veins in the dorso-medial edge of the Wolffian bodies. The lymphatics which grow from the ventral surface of the mesonephritic veins make the large retroperitoneal or præ-aortic sac; those which grow along the dorso-medial edge of the Wolffian bodies, lateral to the aorta, form the iliac sacs, and those which grow dorsal to the aorta make the cisterna chyli and thoracic duct. The thoracic duct is formed in small part from a duct from the left jugular sac; in larger part from a plexus which surrounds the aorta from the cisterna chyli. The retroperitoneal sac drains the diaphragm and the abdominal viscera, except the permanent kidney; the iliac sacs drain the permanent kidney, the abdominal walls, the hindlegs, tail and embryonic membranes.

B. THE JUGULAR SACS.

As has already been stated in part III, the spreading of the primary superficial lymphatic capillaries in the skin of the embryo pig takes place while the embryo is growing from 20 to 50 mm. long. The first lymphatic buds are found when the embryo is about 11 mm. long (fig. 6); the sac is well formed at 18 mm. (fig. 7), and reaches its maximum size in an embryo 30 mm. long (fig. 8). It remains as a large sac until the embryo measures 50 mm., when it begins to be transformed in part into lymph glands (fig. 16).

An understanding of the development of the jugular sac depends on a knowledge of the veins of the region. Indeed, I was not able to inject the early stages until I had so mastered the pattern of the blood vessels that a little blood in any of them enabled me to reproduce the whole picture and so avoid the blood vessels.

The study of the form of the early anterior cardinal vein is shown for the chick by Evans (34) in his figs. 1 to 3. These figures show that the primitive deep dorsal segmental branches drain the ventrolateral surface of the spinal cord. They bring out the gradual curve of the anterior cardinal vein and the more marked bend of the posterior cardinal vein, where the two join the duct of Cuvier. This stage is shown for the chick in cross section by Evans (35) in his fig. 437 B, as well as in (Evans 34) his fig. 4.

In fig. 4 is given an injection of the blood vessels in an embryo pig 7 mm. long, a stage just before the lymphatics begin. The curve of the dorsal border of the cardinal veins toward the duct of Cuvier is

slight, not as marked as in the chick, nor as it is on the left side of this same embryo.

Arising from the cardinal veins there is a series of seven deep segmental branches extending to the nervous system between the area of the primitive posterior cerebral vein (Mall 143), which arches

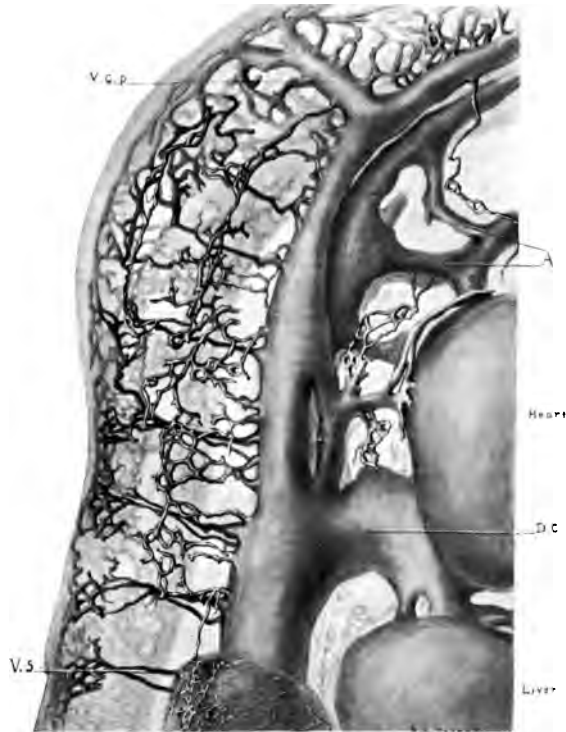


FIG. 4.—Injected specimen to show the anterior cardinal vein and its branches in an embryo pig which measured 7 mm. in its greatest diameter. Magnified about 28 times. This is a stage before the lymphatic vessels have begun. A = aorta arches; D. C. = duct of Cuvier; V. c. p. = vena cerebialis posterior; V. 5 = fifth segmental vein.

downward over the medulla and cord and the cerebral end of the arm bud. The first two of these branches drain the medulla, and hence there are five spinal or segmental branches proper. Each of these branches has a corresponding artery which lies slightly caudalward and nearer the midline. Only the lower four segmental arteries are injected in this specimen. The relative position of artery and vein

shows in fig. 437 (Evans 35). Each of the five spinal veins drains a plexus of wide capillaries, which marks the presence of the developing spinal ganglia.

It is, however, the superficial veins which are of more interest from the standpoint of the lymphatic system. Along the dorso-lateral border of the cardinal veins there is a series of intersegmental sprouts, varying from one to four or five in each interspace. These sprouts drain a superficial capillary plexus which is developing in a groove in the neck which is a continuation of the groove dorsal to the Wolffian ridge. The arteries for this superficial capillary plexus come from the deep segmental arteries (Evans 35, fig. 437). The connections of the plexus with the cardinal vein are most abundant near the posterior cerebral vein. This superficial capillary plexus of the body wall has a most interesting development. For the area between the limb buds it has been worked out by Dr. Helen W. Smith (141), whose figures are copied by Evans (35). This superficial plexus in the tail region shows a constant zone of widened capillaries (35, fig. 467) corresponding to the position of the posterior lymph hearts in birds. For the neck region it is necessary to know this plexus in order to locate the jugular lymphatics, and therefore its development will be followed in further stages.

The ventral branches of the anterior cardinal vein are represented at this stage by one large pericardial vein, which receives branches from the gill arch region. The common stem for the branchial and pericardial vessels connects with the anterior cardinal vein, but it drains directly into the duct of Cuvier (see Grosser and F. T. Lewis as quoted by Evans 35, p. 660) on the right side of this embryo, while on the left side of the same specimen it opens into the anterior cardinal vein.

The earliest lymphatic buds have been found in embryo pigs measuring from 10 to 11 mm. long. All of my measurements mean the greatest length and are made on the fresh specimen. The stage of the first lymphatics is shown in two figures, fig. 5, a total mount of an embryo injected with India ink through the umbilical artery in a specimen 11 mm. long, and fig. 6, a cross section 250 μ thick, through the lymphatic area in an embryo measuring 11 mm. The blood vessels were injected with India ink, and the lymphatics are filled with blood.

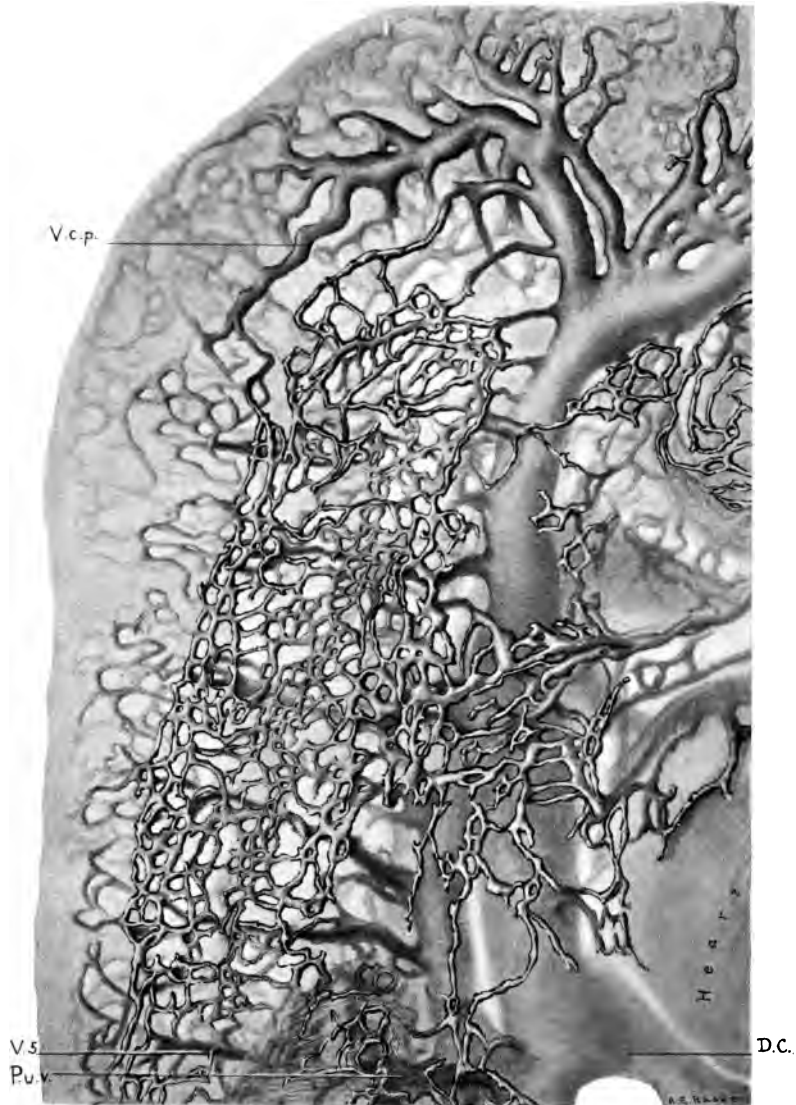


FIG. 5.—Injected specimen of the anterior cardinal vein and its branches in an embryo pig measuring 11. mm. in its greatest diameter. Magnified about 33 times. This is the stage at which the lymphatic vessels begin to develop. The vessels were injected with India ink through the umbilical artery. D. C. = duct of Cuvier; V. c. p. = vena cerebialis posterior; F. u. v. = primitive vena ulnaris; V. 5 = fifth segmental vein.

The blood vessels show a great development. The five spinal segmental veins are clear, draining the large plexuses around each spinal ganglion; the ganglia are now farther ventralward than in the preceding stage. The superficial capillary plexus shows the most marked changes. There is a continuous sheet of superficial capillaries in the

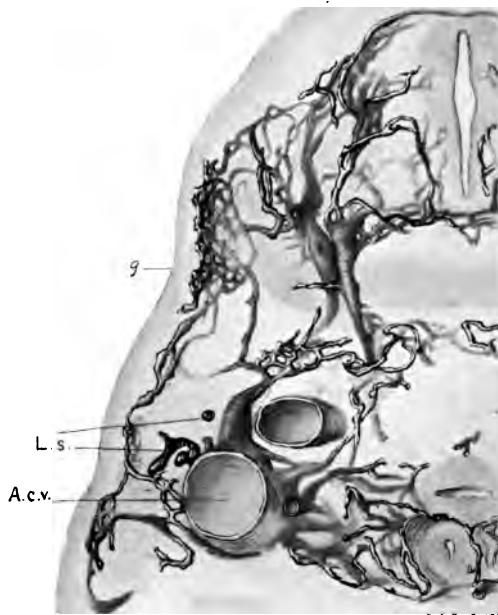


FIG. 6.—Section through the third segmental vein in an injected embryo pig which measured 11 mm. in its greatest diameter. The section, which is $250\ \mu$ thick, shows the relation of the jugular lymphatic sprouts or buds to an anterior cardinal vein. Magnified about 40 times. The blood vessels were injected with India ink through the umbilical artery. The lymphatic sprouts, L. s., have a natural injection of blood; these lymphatic sprouts lie in a non-blood-vascular zone dorso-lateral to the anterior cardinal vein. A. c. v. = vena cardinalis anterior; g = groove.

neck, anastomosing with the branchial vessels, with the pericardial vessels and with the abundant capillary plexus of the arm bud. Opposite the medulla the connections of this superficial plexus with the anterior cardinal vein are very numerous, while opposite the heart a few veins connect it either directly with the anterior cardinal vein or more frequently with the main segmental branches. It is this

zone, opposite the third, fourth and fifth segmental veins, that is the lymphatic area.

To locate the lymphatic area definitely it is necessary to note the details of the superficial veins more carefully. The superficial plexus along the lateral groove is finer meshed and of smaller capillaries than the rest. The groove is an important land mark and it shows best in the cross section, fig. 6. From the plexus in the groove a sheet of wide capillaries is growing dorsalward external to the myotomes, and loops from this sheet connect with the deep plexus around the spinal cord. The skin over the entire dorsal surface of the cord is an entirely non-vascular area at this stage. From the ventral border of the superficial plexus in the groove a sheet of wider and more open-meshed capillaries covers the surface of the embryo over the anterior cardinal vein. The surface contour of this, the lymphatic area, is a swelling seen in fig. 6, which I shall call the jugular lymphatic ridge. The blood capillaries of this area are very superficial and drain into the cardinal vein in four ways: (1) Through the plexus in the groove and its dorso-lateral superficial veins; (2) in small part through direct branches to the lateral surface of the cardinal vein; (3) through branches of the ventro-lateral surface of the anterior cardinal vein which drain the pericardium (fig. 5); and (4) through anastomoses with the capillaries (fig. 5) of the primitive ulnar vein. The primitive ulnar vein has developed from the diffuse capillary arm bud plexus of the preceding stage. It enters the lateral surface of the posterior cardinal vein directly opposite the fifth segmental branch. Thus a part of the blood of the branchial region and of the pericardium drains through the superficial plexus over the lymphatic ridge into the primitive ulnar vein. In a little older embryos, namely, those 12 mm. long, a second vein has developed from the arm bud plexus just cerebralward to the primitive ulnar, which now receives the blood from the plexus of the lymphatic ridge. The complete description of this superficial plexus involves a study of the origin of the external jugular vein which I do not wish to go into until I can illustrate it adequately. I bring up the point here because A. H. Clark (24) has shown that occasionally a lymph trunk in the neck in older pigs opens directly into the external jugular vein near its point of union with the internal jugular vein. It is therefore of importance to note that the capillary plexus in the arm bud, from which this part of the internal jugular vein comes, is present in the embryo when the lymphatics are

budding out, though the internal jugular vein as a whole is formed considerably later.

For the present the interest in the superficial blood capillary plexus is that it covers the lymphatic ridge. It will be seen in fig. 6 that the lymphatic ridge includes a non-vascular area. This non-vascular area is bounded by the anterior cardinal vein and its dorsal branches, the plexus of the groove, the wide-meshed superficial plexus and the ventro-lateral branches of the anterior cardinal vein. An occasional direct lateral branch of the cardinal vein cuts through the otherwise non-vascular area. Within this area are seen the blood-filled lymphatic buds. They lie in the angle between the anterior cardinal vein and its dorso-lateral branches and connect with both. They are opposite the third, fourth and fifth dorsal segmental veins, and extend a distance of 1.5 mm. from the primitive ulnar along the anterior cardinal vein. The tiny lymphatic buds are already sending sprouts away from the parent vein.

The presence of stagnant blood, which has proved so valuable a criterion in the living embryo, must be used guardedly in studying sections, unless there is a complete vascular injection. In the lymphatic area I have noted in the fresh embryo that the blood capillary plexus of the groove is often empty, while the superficial plexus over the lymphatic area and over the pericardium tend to retain some blood. The blood vessels of an area where lymphatics are budding must be thoroughly known before one can be sure of the lymphatic buds. The early lymphatic buds are packed with blood to an extent not common for the veins, which perhaps shows best in figs. 490 and 491 (Sabin 134), for the jugular lymphatic buds in a human embryo.

I have not yet succeeded in injecting the first jugular lymphatic buds in the pig as E. L. Clark has done for the early stages of the posterior lymph heart of the chick. In the embryo 12 mm. long they can be seen in the fresh embryo, and sections show that they have formed a plexus along the margin of the anterior cardinal vein. To see the lymphatic buds at 12 mm. the embryo should be placed in warm Locke's solution while the heart is still beating, and it should be viewed under the high powers of the binocular microscope with the direct sunlight focused upon it. The lymphatic area is wider in the dorso-ventral direction than in fig. 6, but its dorsal boundary is clearly marked by the surface groove. If the superficial blood capillary plexus is empty the lymphatics can then be seen looking like a cluster

of dull red grapes against the cardinal vein; in the older specimens some of the buds are markedly larger than others, while at 14 mm. the largest of them makes a definite sac with large sprouts projecting dorsalward. At this stage, namely, 14 mm., the sac can be injected

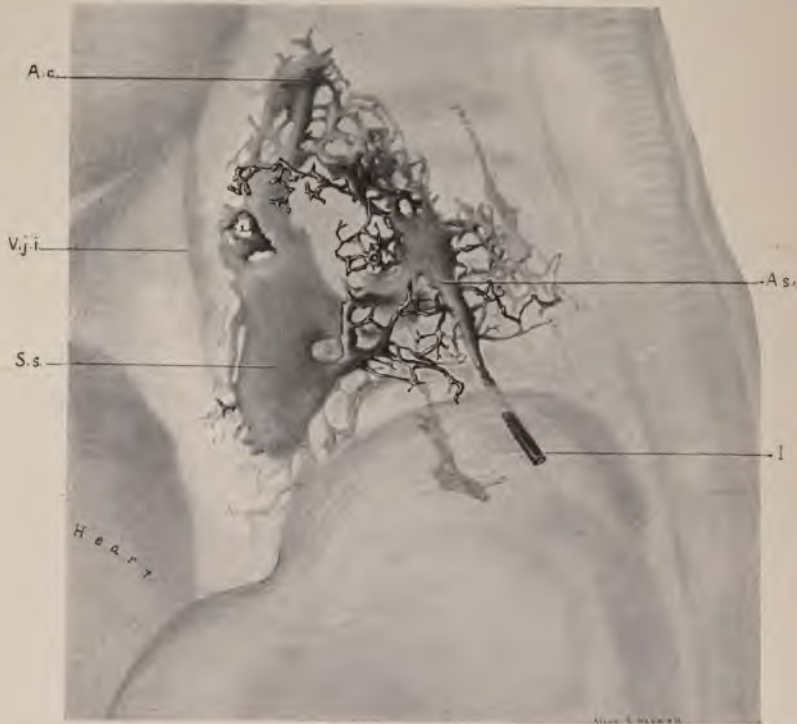


FIG. 7.—Injection of the left jugular lymph sac in an embryo pig which measured 18 mm. in its greatest diameter. The specimen was fixed in 10 per cent formol and cleared by the Spalteholz method. Magnified about 30 times. The glass canula which shows piercing the upper border of the arm bud shows the point of injection. A. c. = anterior curvature of the lymph sac; A. s. = apex of the lymph sac; I. = glass canula used for injection; S. s. = stalk of the lymph sac; V. j. i. = vena jugular interna.

by direct puncture. The preceding or plexus stage is shown by E. L. Clark for the corresponding sac in the chick in fig. 15. The beginning sac is shown for the human embryo in fig. 491 (Sabin 134).

It is not easy to get perfect injections by direct puncture, but by the time the embryo is 16 mm. the dorsal sprouts from the sac are

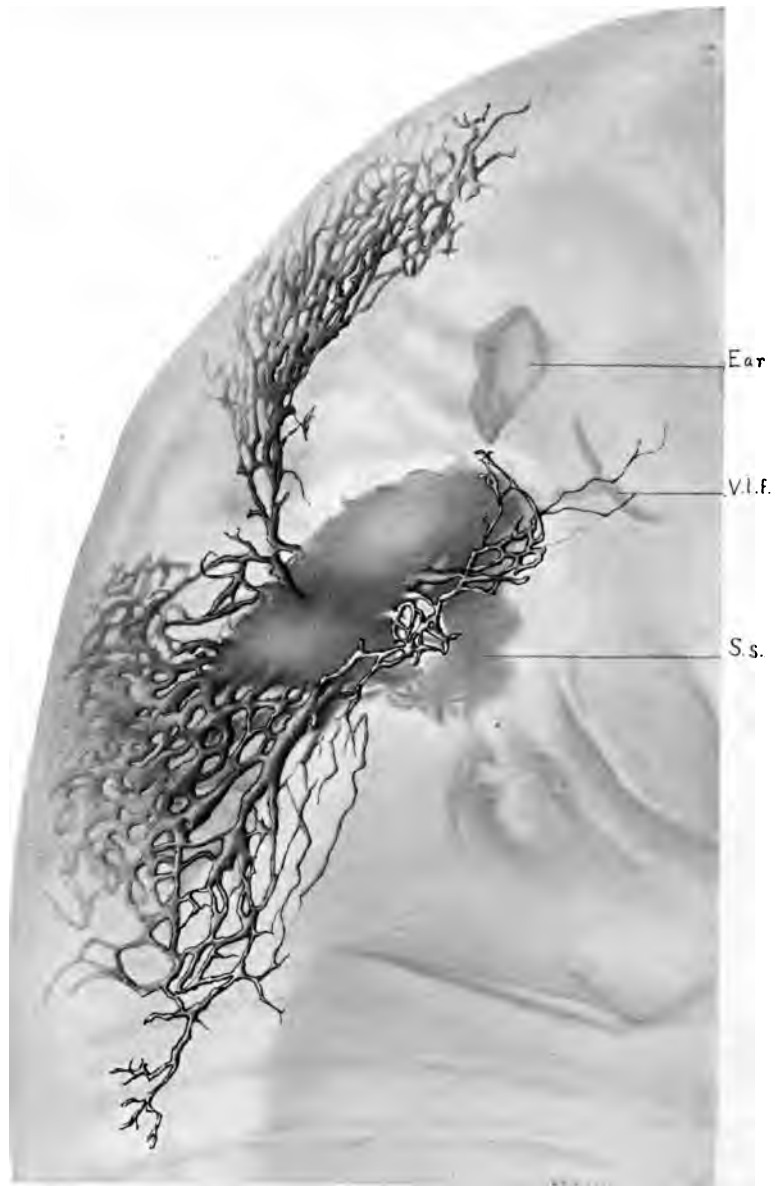


FIG. 8.—Injection of the right jugular lymph sac in foetal pig measuring 3.5 cm. Magnified about 10 times. After A. H. Clark (24); this specimen was shown as fig. 3 in 130; it has since been cleared by the Spalteholz method so that it shows the relation of the superficial lymph vessels to the jugular sac. It shows a complete injection of the suprascapular and occipital plexuses, but an incomplete injection of the superficial cervical plexus. S. s. = the stalk of the sac showing faintly through the shoulder; V. l. f. = vena linguofacialis.

sufficiently large so that the sac can be injected indirectly through them.

The process by which a lymphatic plexus becomes a sac has been called confluence or cavernization by Ranvier (117-118). He says that when two lymph vessels lie in contact the endothelial wall between them disappears (possibly it is retracted) and thus large sacs develop. It is exceedingly interesting that this process does not take place when lymphatic endothelium rests on venous endothelium, as is the case of the jugular lymph sac.

The most interesting stage in the formation of the jugular sac is shown in fig. 7 from an embryo 18 mm. long. To inject the sac at this stage it is again important to note the plan of the blood capillaries. In fig. 6 it will be noted that there is a tiny blood capillary which cuts across the lymphatic area from the superficial plexus to the deeper veins and divides the lymphatic area into two parts, a ventral jugular part and a dorsal part. By the time the embryo is from 16-20 mm. long the path of this slender vessel is occupied by a considerable plexus of blood vessels and by nerves as well. The sprouts from the main jugular sac (fig. 7) have grown up into the dorsal lymphatic area and there developed into an abundant plexus. It is this plexus which can be injected, as is shown by the glass tube which pierces the arm bud. The original sac is shown just lateral to the shadow of the internal jugular vein. This is difficult to inject by direct puncture, because the blood capillary plexus over it has become double and is excessively abundant. Moreover the sac is now just mesial to the developing external jugular vein and lies very close to it. The blood capillary plexus of the groove shown in fig. 6 is now deeper in, and the entire area of the groove so dense a vascular area that no canula can enter the zone without filling the blood capillaries. But just ventral to the groove in the dorsal lymphatic area almost every puncture will fill the lymphatics, provided it avoids the superficial blood capillaries. This dorsal lymphatic area is destined to be the posterior triangle of the neck; even in this early stage it looks translucent and continues to do so, for it is always uncovered by muscle, lying between the trapezius and the sternocleidomastoid muscles (fig. 17).

A description of the form of the sac in fig. 7 will make the basis for the description of the peripheral lymphatics of the head, neck and thorax worked out by A. H. Clark (24) and given in section V. The primitive sac is the portion above the arm bud and lateral to the

internal jugular vein. This becomes the internal jugular trunk; the cerebral end of this portion is an abundant lymphatic plexus, the anlage of a single gland in the pig (A. H. Clark) which drains the pharynx. In the dorsal lymphatic zone the rest of the sac is a complete arch from which develop the lymphatics of the shoulder, head, neck, face, arm and thorax. The dorsal arch of the sac becomes by far its largest portion, as will be readily seen in fig. 8, from an embryo 3.5 cm. long. This is the same specimen shown as fig. 3 (Sabin 130). It has since been cleared by the Spalteholz method, so that it shows the sac as well as the superficial lymphatics.

The transition stages between fig. 7 and fig. 8 are very easy to inject and can be readily imagined. The further development of the sac, especially with reference to the lymphatics along the external jugular vein, is brought out in the sections on the peripheral lymphatics and glands.

C. THE RENAL SACS.

The study of the rest of the lymph sacs in the pig may well begin with the interesting and valuable work of Silvester (140). Silvester has shown that in the monkey all of the lumbar and mesenteric lymphatic ducts drain not by the thoracic duct into the jugular veins, but directly into the renal veins or the surface of the inferior vena cava near the renal veins. This he shows in a number of figures from beautiful injections.

A. RETROPERITONEAL SAC.

It had previously been discovered by Lewis (76) in a study of rabbit embryos that a lymphatic sac develops just ventral to the median mesonephritic vein. Baetjer (8) proved that this, the retroperitoneal or præ-aortic sac, arises from the ventral surface of the large vein which connects the two Wolffian bodies in embryo pigs measuring from 17 to 23 mm. long. Baetjer's fig. 3, from an embryo 19 mm. long, shows the blood-filled lymphatic buds which have been slightly injected from the veins. These buds rapidly form a large sac which lies in the root of the mesentery and is the place of origin for all of the mesenteric lymphatics (Heuer, 43); those that grow to the stomach and intestine, the liver, the capsule of the Wolffian bodies and the reproductive glands. The blood-filled lymphatic buds can be seen in the fresh embryo pig 19 to 20 mm. long, if the intestine is pushed to the

side and the veins are emptied of blood by injecting them with salt solution.

The retroperitoneal sac becomes the largest of all the lymphatic sacs in the pig. As shown in Heuer's fig. 3 (43) it spreads out just behind the rectum and covers the entire area in the root of the mesentery between the two Wolffian bodies. It spreads over the ventral surface of the Wolffian bodies as far as the edge of the reproductive glands. It was from injecting this sac that Heuer was able to study the development of the lymphatics of the intestine.

This sac would give an excellent opportunity to study the process of cavernization. Injections of it with silver nitrate give beautiful specimens, showing the endothelial-covered trabeculæ that cross its lumen. Total preparations of the silvered sac remind one of the trabeculæ in the wall of the cavity of the heart. These trabeculæ, which are the beginning of the process of transferring the sacs into lymph glands, show especially well in sagittal sections of pig embryos 20-25 mm. long.

2. ILIAC SAC—CISTERNA CHYLI.

I can now bring some evidence to show that the iliac lymphatics which drain the legs, tail and abdominal wall, and the cisterna chyli which forms the lower part of the thoracic duct, arise together as buds from veins of the Wolffian bodies. A complete account of this process needs a more extensive illustration of the blood vessels of the region than I can give at this time. In the pig the lymphatics which bud off from the veins of the Wolffian body and grow forward dorsal to the aorta to form the cisterna chyli and caudalward along the edge of the Wolffian body to form the iliac lymphatics, do not begin until the embryo is 22 mm. long. I found that they arise from the mesonephritic veins because a direct puncture of the blood-filled buds entered the main veins and not the blood capillaries. In a litter of pigs which measured 23 mm. I washed out the blood vessels with Locke's solution, and then opened the specimens and pushed one of the Wolffian bodies over toward the midline. A plexus of blood-filled lymphatics was then readily seen dorsal to the aorta. I succeeded in puncturing some of the larger vessels of the plexus forming the cisterna chyli in three specimens and saw the ink run from the lymphatics into the mesonephritic veins. In all of these injections the cisterna chyli is obscured by extravasations at the point of injection, but they all show the

iliac lymphatics. For the present I shall describe the renal lymphatics from sections which can be done in the blood-packed stage.

The description of the iliac lymphatics, however, needs an outline of the blood vessels of the region. If the veins of the prevertebral space between the level of the median vein connecting the Wolffian bodies and the root of the tail be injected it will prove that they

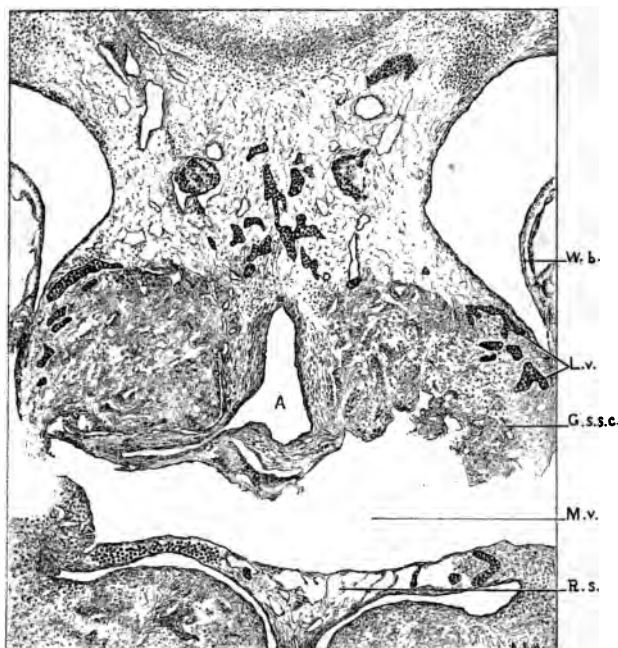


FIG. 9.—Section through the hilus of the Wolffian bodies to show the place of origin of the iliac lymphatic vessels and the abdominal part of the thoracic duct in an embryo pig 23 mm. long. (Specimen 23b.) Magnified 50 times. A. = aorta; G. s. s. c. = gl. suprarenalis s. corticalis; L. v. = lymph vessels filled with blood; M. v. = vena mesonephritica; R. s. = retroperitoneal lymph sac, a part of which is filled with blood, a part empty; W. b. = edge of the Wolffian body.

are enormously abundant. In the embryo measuring 20 mm. the segmental veins draining the cord and the body wall make an extensive network, which drains in part into the median vein of the Wolffian body and thence directly to the vena cava, while farther caudalward this same plexus drains directly into the large surface

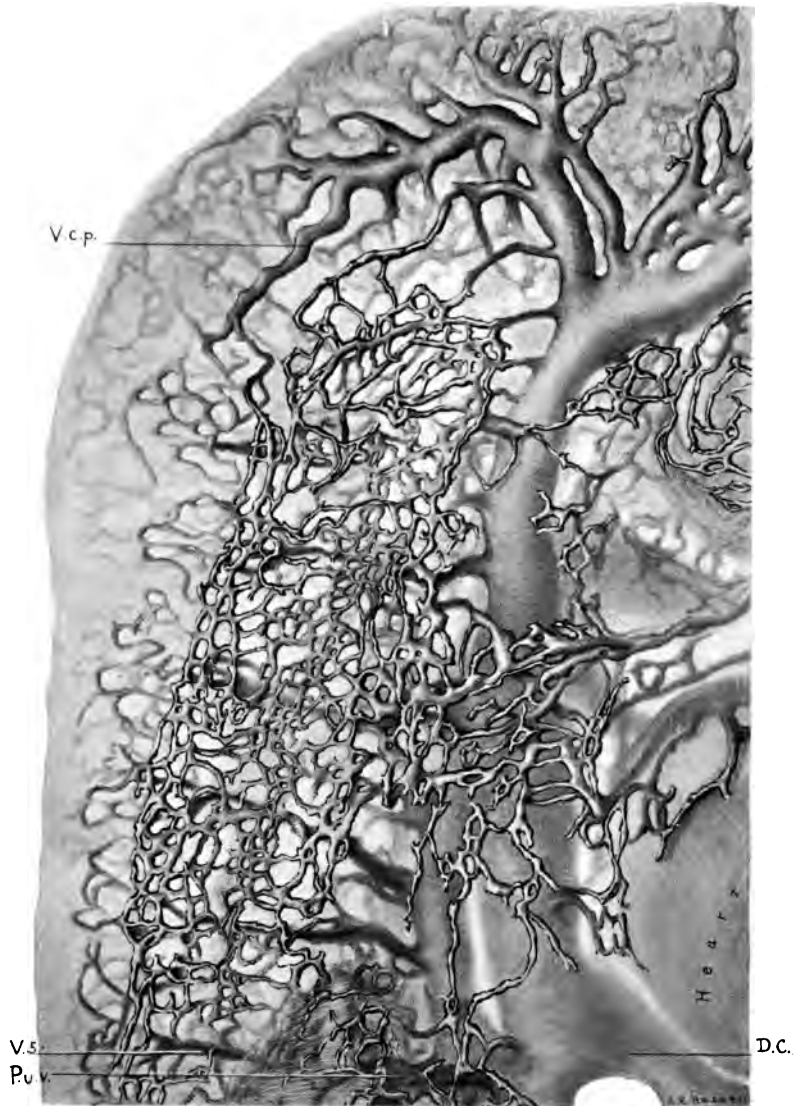


FIG. 5.—Injected specimen of the anterior cardinal vein and its branches in an embryo pig measuring 11. mm. in its greatest diameter. Magnified about 33 times. This is the stage at which the lymphatic vessels begin to develop. The vessels were injected with India ink through the umbilical artery. D. C. = duct of Cuvier; V. c. p. = vena cerebialis posterior; F. u. v. = primitive vena ulnaris; V. 5 = fifth segmental vein.

The blood vessels show a great development. The five spinal segmental veins are clear, draining the large plexuses around each spinal ganglion; the ganglia are now farther ventralward than in the preceding stage. The superficial capillary plexus shows the most marked changes. There is a continuous sheet of superficial capillaries in the

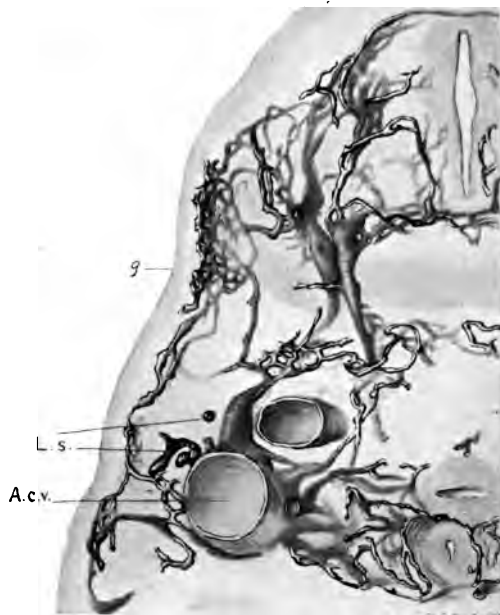


FIG. 6.—Section through the third segmental vein in an injected embryo pig which measured 11 mm. in its greatest diameter. The section, which is $250\ \mu$ thick, shows the relation of the jugular lymphatic sprouts or buds to an anterior cardinal vein. Magnified about 40 times. The blood vessels were injected with India ink through the umbilical artery. The lymphatic sprouts, L. s., have a natural injection of blood; these lymphatic sprouts lie in a non-blood-vascular zone dorso-lateral to the anterior cardinal vein. A. c. v. = vena cardinalis anterior; g = groove.

neck, anastomising with the branchial vessels, with the pericardial vessels and with the abundant capillary plexus of the arm bud. Opposite the medulla the connections of this superficial plexus with the anterior cardinal vein are very numerous, while opposite the heart a few veins connect it either directly with the anterior cardinal vein or more frequently with the main segmental branches. It is this

zone, opposite the third, fourth and fifth segmental veins, that is the lymphatic area.

To locate the lymphatic area definitely it is necessary to note the details of the superficial veins more carefully. The superficial plexus along the lateral groove is finer meshed and of smaller capillaries than the rest. The groove is an important land mark and it shows best in the cross section, fig. 6. From the plexus in the groove a sheet of wide capillaries is growing dorsalward external to the myotomes, and loops from this sheet connect with the deep plexus around the spinal cord. The skin over the entire dorsal surface of the cord is an entirely non-vascular area at this stage. From the ventral border of the superficial plexus in the groove a sheet of wider and more open-meshed capillaries covers the surface of the embryo over the anterior cardinal vein. The surface contour of this, the lymphatic area, is a swelling seen in fig. 6, which I shall call the jugular lymphatic ridge. The blood capillaries of this area are very superficial and drain into the cardinal vein in four ways: (1) Through the plexus in the groove and its dorso-lateral superficial veins; (2) in small part through direct branches to the lateral surface of the cardinal vein; (3) through branches of the ventro-lateral surface of the anterior cardinal vein which drain the pericardium (fig. 5); and (4) through anastomoses with the capillaries (fig. 5) of the primitive ulnar vein. The primitive ulnar vein has developed from the diffuse capillary arm bud plexus of the preceding stage. It enters the lateral surface of the posterior cardinal vein directly opposite the fifth segmental branch. Thus a part of the blood of the branchial region and of the pericardium drains through the superficial plexus over the lymphatic ridge into the primitive ulnar vein. In a little older embryos, namely, those 12 mm. long, a second vein has developed from the arm bud plexus just cerebralward to the primitive ulnar, which now receives the blood from the plexus of the lymphatic ridge. The complete description of this superficial plexus involves a study of the origin of the external jugular vein which I do not wish to go into until I can illustrate it adequately. I bring up the point here because A. H. Clark (24) has shown that occasionally a lymph trunk in the neck in older pigs opens directly into the external jugular vein near its point of union with the internal jugular vein. It is therefore of importance to note that the capillary plexus in the arm bud, from which this part of the internal jugular vein comes, is present in the embryo when the lymphatics are

budding out, though the internal jugular vein as a whole is formed considerably later.

For the present the interest in the superficial blood capillary plexus is that it covers the lymphatic ridge. It will be seen in fig. 6 that the lymphatic ridge includes a non-vascular area. This non-vascular area is bounded by the anterior cardinal vein and its dorsal branches, the plexus of the groove, the wide-meshed superficial plexus and the ventro-lateral branches of the anterior cardinal vein. An occasional direct lateral branch of the cardinal vein cuts through the otherwise non-vascular area. Within this area are seen the blood-filled lymphatic buds. They lie in the angle between the anterior cardinal vein and its dorso-lateral branches and connect with both. They are opposite the third, fourth and fifth dorsal segmental veins, and extend a distance of 1.5 mm. from the primitive ulnar along the anterior cardinal vein. The tiny lymphatic buds are already sending sprouts away from the parent vein.

The presence of stagnant blood, which has proved so valuable a criterion in the living embryo, must be used guardedly in studying sections, unless there is a complete vascular injection. In the lymphatic area I have noted in the fresh embryo that the blood capillary plexus of the groove is often empty, while the superficial plexus over the lymphatic area and over the pericardium tend to retain some blood. The blood vessels of an area where lymphatics are budding must be thoroughly known before one can be sure of the lymphatic buds. The early lymphatic buds are packed with blood to an extent not common for the veins, which perhaps shows best in figs. 490 and 491 (Sabin 134), for the jugular lymphatic buds in a human embryo.

I have not yet succeeded in injecting the first jugular lymphatic buds in the pig as E. L. Clark has done for the early stages of the posterior lymph heart of the chick. In the embryo 12 mm. long they can be seen in the fresh embryo, and sections show that they have formed a plexus along the margin of the anterior cardinal vein. To see the lymphatic buds at 12 mm. the embryo should be placed in warm Locke's solution while the heart is still beating, and it should be viewed under the high powers of the binocular microscope with the direct sunlight focused upon it. The lymphatic area is wider in the dorso-ventral direction than in fig. 6, but its dorsal boundary is clearly marked by the surface groove. If the superficial blood capillary plexus is empty the lymphatics can then be seen looking like a cluster

the time, and he has published, with my permission, a valuable reconstruction of my specimen in the *Anatomical Record* for 1912 (Kampmeier 66).

The thoracic duct needs to be considered in relation to the veins of the region. Fig. 11 is a section through the seventh cervical vertebra of an embryo pig 19 mm. long, which is before the thoracic duct begins. There is a complete vascular injection. It will be seen that there is a vascular zone ventral to the vertebra, and from this zone veins surround the sympathetic nerves and enter the dorso-medial border of the internal jugular vein. The esophagus has a plexus of blood vessels in the submucosa, but dorsal to the esophagus is a non-vascular zone of loose connective tissue. The jugular stem of the thoracic duct grows into this non-vascular area dorsal to the esophagus, as is shown in fig. 12 from 23a. In this tracing of the section the veins are arbitrarily made black and the lymphatics are shown empty, though in the section the left lymphatic sac and its ducts were injected. The thoracic duct lies in the margin of the vascular zone. As Kampmeier's reconstruction of this specimen shows, there is a considerable plexus of lymphatics dorsal to the esophagus near their place of origin from the jugular sac. This plexus connects with the left jugular sac in three places. From the injected plexus a short duct follows the left cardinal vein and I think grows to the heart and lungs. A longer vessel crosses to the right side and is the jugular segment of the thoracic duct. This crossing of the duct behind the aorta I have frequently, though not always, noted in older stages.

The right lymphatic duct curves ventralward and grows to the heart and lungs. Its course is shown in fig. 13 for an embryo 25 mm. long. The asymmetry of the thoracic duct is confined to the jugular portion and corresponds with the asymmetry of the aorta. It is interesting to note that Sala (137, Taf. 14, Fig. 16) and Pensa (104, Taf. 15, Fig. 2) picture a symmetrical thoracic duct in the bird.

The position of the cisterna chyli has already been shown in figures 9 and 10. The cisterna chyli and lower part of the thoracic duct arise in common with the iliac sacs from the mesonephritic veins on either side, as shown in fig. 9. These lymphatic buds from the two sides meet dorsal to the aorta and grow both cerebralward and caudalward along the dorsal wall of the aorta. This makes an abundant plexus of blood-filled lymphatics along the course of the abdominal aorta. Any sections of pig embryos of this stage will show that the

veins around the aorta are very abundant, so that the non-vascular space is small. Just opposite the adrenal anlage, however, there is a transition zone which is less vascular. It marks the end of the azygos veins. Below this level the segmental veins drain through the Wolffian bodies, and here the lymphatics form a wider plexus, which becomes the cisterna chyli, fig. 10. As in the adult there is a gradual tran-

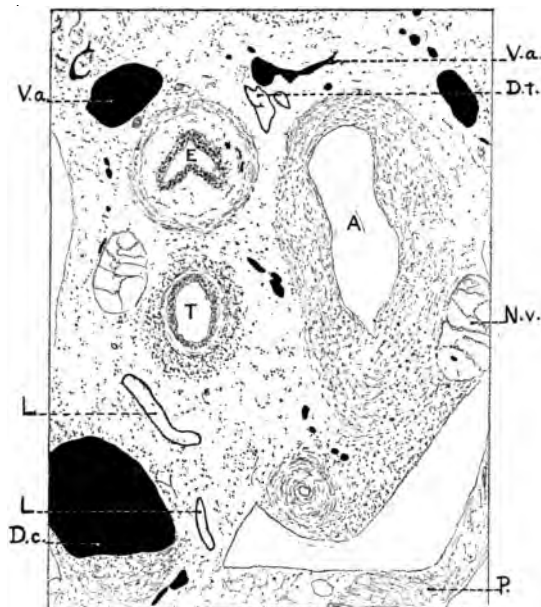


FIG. 13.—Section through the upper thoracic region of an embryo pig measuring 25 mm. in which the blood vessels are injected, to show the vessels of the right lymphatic duct approaching the heart. Magnified 40 times. The injection of the blood vessels was made through the umbilical artery. A. = aorta; D. C. = duct of Cuvier; D. t. = ductus thoracicus; E. = esophagus; L. = lymph vessels to the heart from the right lymphatic duct; N. v. = nervus vagus; P. = pericardium; T. = trachea; V. a. = vena azygos.

sition between the cisterna chyli and the rest of the aortic plexus, both the part which extends caudalward and the part which extends cerebralward. Figure 10 shows the cisterna chyli while it is still a plexus and not a sac. The series shows that in many places this dorsal plexus of lymphatics is sending sprouts around to the ventral præ-aortic sac. All of the abdominal sacs, the retroperitoneal, ventral to the aorta, the two lateral iliac sacs and the dorsal plexus connect with

each other. In following the series cerebralward from the level of fig. 9 it is clear that the blood-filled lymphatics not only arch across the midline dorsal to the aorta, but they follow the border of the Wolffian bodies lateral to the aorta. Above the adrenal bodies the Wolffian bodies approach very close to the aorta, and some of the lymphatic buds curve around the ventral surface of the aorta. Thus in a specimen 23 mm. long there is a plexus of blood-filled lymphatics surrounding the aorta and extending into the lower thorax as far as the cerebral pole of the Wolffian bodies. At the cerebral end of the Wolffian bodies there are large veins which curve ventral to the aorta and connect the veins of the capsule of the Wolffian bodies. Some of the lymphatics are near these median veins.

The finding of the renal lymphatics in the blood-packed stage makes it quite certain that the thoracic duct arises in two places: First, the jugular stem, which can be injected from the left jugular sac and develops long after the jugular sacs are entirely empty of blood; and, secondly, the renal plexus, which surrounds the aorta and forms a definite cisterna chyli.

These two observations, namely, (1) the presence of blood in one part and its absence in the other, and (2) the fact that injections prove a connection of the jugular stem with the jugular sac, and of the abdominal plexus with the mesonephritic veins, make it quite certain that the two portions of the thoracic duct are distinct from each other in embryo pigs measuring 23 mm., and hence that the thoracic duct begins in two places. Practically the entire question at issue between those of us who think that the lymphatics grow by their own endothelial wall and those who do not centers around the question of how the thoracic duct develops, or, to put it more specifically, how these two lymphatic anlagen become connected. The discussion centers around my specimen 23a, which Kampmeier studied and which he uses as his most conclusive evidence of the theory of the growth of lymphatics by the addition of tissue spaces. On the other hand, it is my theory that the thoracic duct grows from these two anlagen from the veins by the same method by which other lymphatic capillaries can be seen to grow in a living specimen, namely, by the sprouting of their endothelial wall. It is true that I have not yet sufficiently mastered the difficulties of injecting the renal lymphatics to demonstrate the progression of the thoracic duct and indeed it may not prove possible to get as conclusive proof of the origin of the deep lym-

phatic sacs as of the more superficial ones; yet the inference that they arise in the same manner is in harmony with our modern knowledge of morphology. I wish to postpone a discussion of Kampmeier's evidence until I have described the thoracic duct in a human embryo, and discussed the development of the theories opposed to my own—of which Kampmeier's work is a part (Sec. VIII).

2. PRIMARY LYMPHATIC SYSTEM IN HUMAN EMBRYOS.

On this description of the lymphatic system in one mammalian form I shall base an account of what is known of the primary system in other forms. All of the primary lymphatic sacs have been found in the human embryo. The jugular sac is the earliest to appear. It has been found as a few blood-packed buds in two embryos measuring 8 and 9 mm. (Mall collection, No. 397 and No. 163, Sabin 134.) They are on the lateral surface of the anterior cardinal vein near the duct of Cuvier and were described as veins (Sabin 133, 134), since at that time the blood-filled buds were thought to be blood capillaries. The blood-packed buds extend along the lateral surface of the anterior cardinal vein and very early develop sprouts that project dorsward. These are plain in an embryo measuring 11 mm. (Mall collection, No. 353, figs. 7 and 8, Sabin 133, and figs. 490 and 491, Sabin 134.) The figures of this specimen, together with the reconstruction of Lewis (Harvard collection No. 1000, measuring 16 mm.; No. 189, measuring 11.5 mm.; and No. 1322, measuring 16 mm., figs. 1-3, Lewis 78), show that the jugular lymphatics in the human embryo not only bud from the anterior cardinal veins, but from the posterior cardinal vein, and the common stem or plexus which forms the origin of the primitive ulnar and thoraco-epigastric veins. A large extension of the jugular sac along the primitive ulnar vein is characteristic of human embryos (fig. 12, Sabin 133, or fig. 493, Sabin 134, and figs. 2 and 3 after Lewis 78).

In a human embryo 10.5 mm. long I have found a small sac only partly filled with blood (figs. 3-6, Sabin 133, and figs. 488, 489, Sabin 134). This specimen has also a beginning thoracic duct. These two facts show that there is considerable variation in the rate of growth of the lymphatic system of different specimens.

The study of human embryos has also brought out the fact that the openings of lymphatics into the veins can only be made out when

the section happens to be cut in the right plane for each valve. For the jugular sac frontal sections are the best (figs. 492, 494, Sabin 134).

A new embryo in the Mall collection, No. 460, measuring 21 mm., is of great interest in connection with the renal lymphatics. A careful reconstruction of this specimen with abundant illustrations would be of value, but for the present I can give only a description. I injected the embryo with India ink into the umbilical artery while the heart was still beating. The vascular injection is almost perfect. The embryo was then put directly into bichloride-acetic and the fixation is excellent.

In marked contrast to the embryo pig of about the same length, the Wolffian bodies are disappearing, being pushed caudalward by the growing permanent kidneys. The cerebral pole of the Wolffian bodies lies far to the side opposite the median mesonephritic vein which, as Baetjer (8, fig. 7) shows, connects the Wolffian bodies opposite their hilum in embryo pigs measuring 20 mm. This median vein in the human embryo (No. 460) passes over the ventral surface of the adrenal bodies and still receives the veins of the cerebral pole of the Wolffian bodies. It also receives a plexus of veins from the permanent kidneys. It is markedly asymmetrical on account of the development of the vena cava on the right side.

Blood-filled lymphatic buds completely surround this mesonephritic vein in the midline, making the anlage of the retroperitoneal or præ-aortic sac; some of those that come from the dorsal surface of the vein have pushed between the masses of the sympathetic ganglia and reached the dorsal surface of the aorta. In some sections, the retroperitoneal buds are partly emptied of their blood. The area at the root of the mesentery opposite the median mesonephritic vein is small in the human embryo as compared with the corresponding area in the pig and the retroperitoneal sac is correspondingly small.

The iliac sacs and the beginning thoracic duct are also present. Along the dorso-medial wall of the kidney in the angle between the segmental veins and the plexus of renal veins are two long iliac sacs. They are evidently farther advanced than the retroperitoneal sac, for they are nearly empty. The one on the left side measures 1.8 mm. and extends to the bifurcation of the aorta. On the right side the empty sac is considerably shorter, but its lower part is replaced by blood-filled buds along the inferior vena cava.

In tracing the lymphatics cerebralward, from the level of the hilus of the kidney, there is a small lymphatic capillary plexus in the place of the cisterna chyli and the two lateral sacs become a plexus of small ducts which can be traced the entire length of the permanent kidneys and then along the dorso-lateral surface of the aorta, just ventral to the azygos veins. This small plexus of ducts is nearly empty on the left side, but full of blood on the right side. The two plexuses can be followed almost to the level of the bifurcation of the trachea. Some blood in the plexus on the left side just before the vessels end makes it possible that the renal part of the thoracic duct does not yet connect with the jugular part of the duct.

This particular specimen is interesting in regard to certain zones of dilated tissue spaces which are conspicuous along the aorta. There are some of these large spaces near the bifurcation of the aorta, in the root of the mesentery near the retroperitoneal sac, and along the ventral surface of the aorta, especially opposite the mesentery of the stomach and opposite the bifurcation of the trachea. I have studied them with care to see if they have any definite relation to the developing lymphatics and am sure that they have not. Sometimes they are near the lymphatics and sometimes not. Those in the thorax are not adjacent to the lymphatic plexus. They can be distinguished from the endothelial-lined lymphatics, but if one were convinced that lymphatics came from tissue spaces one might imagine transition pictures between the tissue spaces and the ducts. This is particularly true when the endothelium of a lymphatic vessel sags away from the surrounding tissue, a picture familiar to histologists in sections showing lymphatics in adult tissues. The further discussion of the thoracic duct is in part VIII.

All of the primary lymphatic system is present in a human embryo measuring 30 mm. (Mall collection No. 86, fig. 12, Sabin 133, copied as fig. 493, Stricker 144). This stage represents the maximum size of the jugular sac. The dorsal arch is large and its line of separation from the jugular part is indicated by the perforation for the cervical nerves and blood vessels. The very small mesenteric sac and larger iliac sacs are distinct as well as the complete thoracic duct.

3. PRIMARY LYMPHATIC SYSTEM IN RABBIT EMBRYOS.

In 1906 F. T. Lewis (76) published a valuable paper on the lymphatic system in rabbit embryos. Besides excellent reconstruction of

the primary lymphatic system in rabbits, the cat and the pig, this paper has three important points: First, the discovery that the early lymphatics are filled with blood, which has proved to be of such value, now that its meaning is understood. Second, the discovery of the retroperitoneal sac which he described as lying adjacent to the mesenteric veins. He also showed the iliac sacs and cisterna chyli; all of which we now know come from the renal veins (Sabin 136). Third, he noted that lymphatic ducts when they are reconstructed from sections appear as a row of beads. Since these rows of beads occurred along the veins in his reconstructions and since he could not find the connections of the early sacs with the veins he was led to suggest that lymphatics might arise from multiple anlagen split off from the veins along their course. The apparently isolated endothelial-lined vessels found in serial sections we call Meyer-Lewis anlagen. They are discussed in section VIII.

The jugular sac in rabbits has an early extension along the primitive ulnar vein like that of the human embryo. The dorsal portion of the sac, for the posterior triangle of the neck, arises from a large stalk from the primary jugular portion between the level of the 4th and 5th cervical nerves.

4. PRIMARY LYMPHATIC SYSTEM IN THE CAT.

The primary lymphatic system in the cat will be described from the extensive work of Huntington and McClure (51-58 and 91-94), one reconstruction of Lewis (76) and a few injections of my own. The work of Huntington and McClure has been mainly on the development of the jugular lymph sacs and the thoracic duct in the cat and their observations are for the most part brought together in two extensive papers (54 and 58). Their work is difficult to analyze and cannot be done entirely accurately without seeing their sections. They have relied on the appearances of sections and the appearances of wax models from them to differentiate lymphatics, and since these are not adequate criteria it is impossible to be sure that all the structures shown as lymphatics are lymphatics; in fact, one can be certain that some of them are not. Their theories in regard to the origin and development of the lymphatic system are discussed in sec. VIII. In the article (54) on the jugular lymph sac of the cat figs. 8 to 21 may be disregarded as evidence, since they are diagrams. Figure 22, which they

group under "Early Venous Stages," is, I think, a reconstruction of the cardinal veins before the lymphatics begin (and is therefore to be compared with Evans' (34) fig. 3, and with my fig. 4 in this paper). In their fig. 22 the fourth segmental vessel is shown and described (page 226) as "formed by the confluence of two extended para-neural channels." This is intended to indicate the beginning of the lymphatics, but since their fig. 43 from an embryo 10 mm. long shows undoubted lymphatics in the blood-packed stage, I think it is practically certain that the specimen measuring 5 mm. does not show lymphatics. It is not necessary to prove that veins do not open into the tissue spaces around nerves.

The description of the stages between 5 and 10 mm. is bound up with the discussion of "fenestration." In reconstructing the veins Huntington and McClure have noted the pattern of the developing veins, which is especially well shown in fig. 4, in connection with the pericardial veins. In this specimen the pericardial veins drain into a loop which connects the anterior cardinal vein with the duct of Cuvier. The formation of this loop is a part of the process by which the pericardial veins shift from the duct of Cuvier to the anterior cardinal vein. Numerous small loops are seen along the anterior cardinal veins in figs. 4 and 5. They are characteristic of the veins. They suggested to Huntington and McClure a vague conception of a relation to the lymphatics which they expressed as follows (p. 204 in 54): "In a subsequent stage confluence of these 'fenestræ' results in the more or less complete separation of the primary vein into two parallel secondary channels, which appears to be a uniform principle in the development of parallel venous trunks. Or, as in the case of the jugular lymph sac, further extension of the same process may result in separating from the main venous channels elements which unite to form a closed sac entirely distinct from the vein from which it arose. The term 'fenestration' means, therefore, in the sense in which it is employed in this paper, one of the last stages in the definite crystallization of the venous system out of an indefinite antecedent plexiform condition, and the determination of an important element in lymphatic organization, closely associated with the embryonic venous system." The lymphatics which they thought formed by this process they termed "veno-lymphatics." Both terms "fenestration" and "veno-lymphatics" should drop out; there are no veno-lymphatics, for the lymphatics bud directly from the veins as lymphatics, and fenestration is a term

that describes models better than the actual process of growth by sprouting by which the veins are formed.

Some of the figures of the models from embryos measuring 7 mm., for example fig. 33, represent lymphatics in the early plexus stage; I do not, however, find any mention of the presence of blood. Figure 46 shows the beginning of the enlarging of the plexus into sacs. From their later stages figs. 46 to 65, but much better from Lewis, fig. 6 (76), can be made out the especial characteristics of the jugular sacs in the cat. They are (1) that the jugular sac in the cat arises from the posterior cardinal vein as well as from the anterior cardinal vein. This point is well shown in Huntington and McClure figures. (2) The cerebral end of the ventral or jugular portion of the sac is very large. This is the part that drains the larynx. (3) The internal jugular trunk is small. (4) The dorsal apex of the sac which lies in the posterior triangle of the neck is large. (See Lewis' fig. 6.) It is connected with the cerebral end by a slender vessel. In injections of the sac in embryos between 30 and 60 mm. long it looks very much like the sac in a bovine embryo as shown by Polinski (107) in his fig. 7. It lies nearer the arm than the corresponding portion of the sac in the pig. (5) There is a large extension though possibly a distinct sac (Lewis, fig. 6) along the primitive ulnar and thoraco-epigastric veins. This sac is very conspicuous in older stages, extending into the axilla and receiving large trunks from the thoracic wall. This makes the jugular lymphatic sac in the cat more like the human than the pig (sec. V).

The segmental divisions of the sac as indicated by colors in the Huntington and McClure figures are arbitrary and not as valuable as the divisions I have just given, which correspond to the function of the different glands which develop from the sac.

Lewis' figure also shows a very interesting view of the retroperitoneal and iliac sacs as a continuous plexus, which again emphasizes the fact that the renal sacs anastomose with each other.

5. PRIMARY LYMPHATIC SYSTEM IN BIRDS, POSTERIOR LYMPH HEART AND JUGULAR LYMPH SAC.

The history of our knowledge of the lymphatic system in birds is most interesting. The early discoveries of the posterior lymph hearts by Panizza, A. F. J. Mayer, and Stannius, as well as the work of

Budge on the relation of the heart to the allantoic vessels, have already been given. From that time on there was no advance until the work of Sala (137), who studied the development of the posterior lymph hearts and thoracic duct. He described the posterior lymph hearts as arising in relation to the lateral branches of the first five coccygeal veins, during the seventh day of incubation. We now know that the posterior lymph hearts bud from the veins during the 5th day (E. R. and E. L. Clark, 29), so that Sala was describing the transformation of the plexus into the sac. He noted the connections with the veins but described the sac as arising from excavations in the mesenchyme. His work, published a short time before my paper on the origin of the lymphatic system from the veins (Sabin 129), was an advance over the preceding work, for it placed the first lymphatics near the veins instead of in the periphery. Sala figured the thoracic duct in the bird as a symmetrical structure (Taf. 14, Fig. 16), and this is also shown in Pensa (104, Taf. 15, Fig. 2). Sala described the early stages of the thoracic duct as being solid cords of cells. This observation, which has been confirmed by Mierzewski (96), makes it necessary to restudy the thoracic duct in the chick, which is now possible through the great improvement in our methods of injection.

The most recent work on the lymphatic system in birds has been done by Mierzewski (96), Jolly (59), Miller (97) and E. R. and E. L. Clark (27-29).

I have already brought out the fundamental importance of the Clarks' work, showing that the lymphatics bud off from the veins in a non-vascular zone, and show a continuous growth to the periphery. Mierzewski had shown that the early lymphatics grow along the lateral line of the embryo, as shown in his fig. 3. It was these lymphatics which grow to connect the posterior lymph heart with the jugular lymph sac that E. L. Clark observed in their blood-filled stage and which led her to watch the process of budding in the living embryo.

The process may best be described in a quotation from p. 254 (29):

The first evidence of lymphatics in the tail region of living chicks is the appearance of a number of separate knobs, filled with stagnant blood, a little darker in color than the circulating blood, just lateral to several of the most anterior of the dorsal intersegmental coccygeal veins. The connections with the veins cannot be seen, since the knobs lie between them and the observer, but ink injected into the knobs can be seen to pass directly into the main intersegmental veins. Between the separate knobs no anastomoses can be seen, nor can any be discovered by injection.

Soon after these knobs appear (in about fifty-five minutes), similar ones develop about them which have fine connections with them, thus forming a small cluster. The new ones are located partly on either side of the first and partly superficial to them. Their injection now shows discreet tiny clusters, somewhat like bunches of grapes, connected, as were the earliest knobs, with the same intersegmental veins. These clusters are still separate from one another.

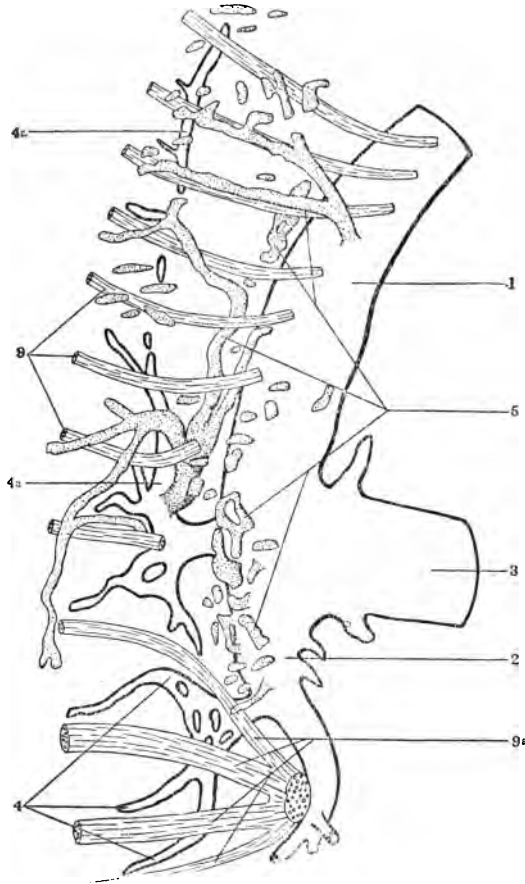


FIG. 14.—After Miller. Diagram from an reconstruction of the veins and nerves of the cervical and upper thoracic regions of a chick embryo 13.5 mm. long, after 5 days and 10 hours of incubation; right side. 1. Præ-cardinal vein. 2. Post-cardinal vein. 3. Duct of Cuvier. 4. Intersegmental (dorsal) veins. 5. Lateral group of vascular islands and veno-lymphatic vessels. 9. Spinal (cervical) nerves. 9a. Brachial plexus. After Miller, *Amer. Jour of Anat.*, 1912, Vol. 12, fig. 12.

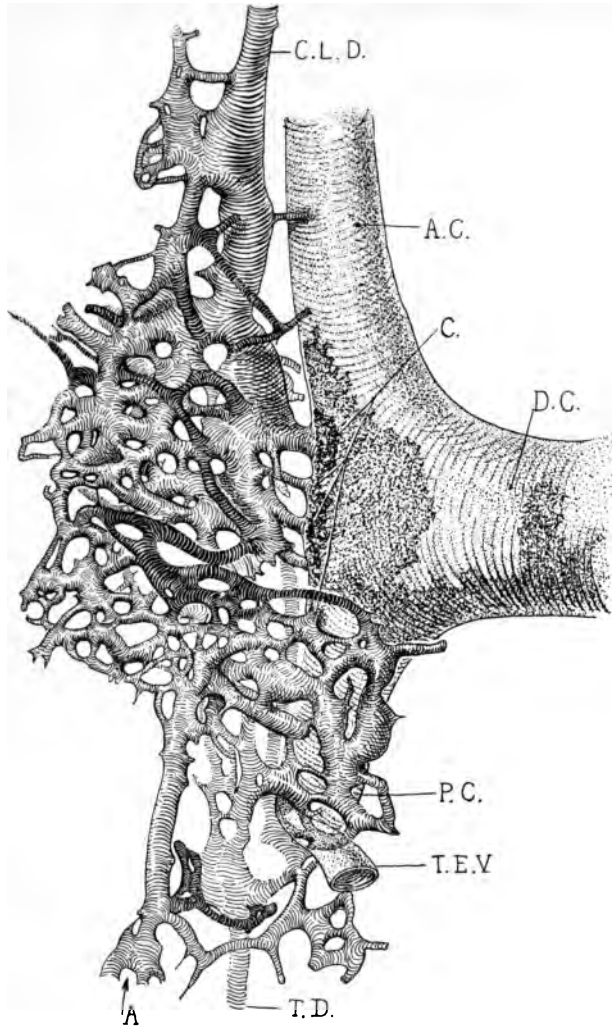


FIG. 15.—After E. L. Clark. Lateral view of the jugular lymphatic plexus of the right side of a chick embryo 14 mm. long after an incubation of 5 days and 20 hours. The measurement was the greatest length and was taken after fixation. With a very fine canula (the lumen of the point being about $15\ \mu$ in diameter) diluted India ink was injected under a binocular microscope into one of the superficial lymphatic capillaries in the skin between the fore and hindlegs. From here the ink filled the deep jugular lymphatic plexus, and from there a few granules entered the vein through five connections (C). The drawing was made with the help of a camera lucida with a Zeiss binocular microscope (oculars No. 4, objective A_3). Magnified 67 times. A. = vessel which connected the superficial lymphatic capillaries with the jugular lymph plexus and which was injected; A. C. = vena cardinalis anterior; P. C. = vena cardinalis posterior; D. C. = duct of Cuvier; C. = communications between the jugular lymphatic plexus and the veins; T. D. = deep lymphatic vessel; T. E. V. = vena thoraco-epigastrica; C. L. D. = cervical lymph vessel. After E. L. Clark's fig. 1, *Anat. Record*, 1912, Vol. 6, p. 263.

There is a rapid extension of these blood-filled structures, and soon, in about an hour and a half after their first appearance, connections between neighboring clusters may be seen. Injection at this stage with India ink shows an anastomizing plexus, connected, as before, with the intersegmental veins. Injection with silver nitrate shows distinct endothelial markings in the walls of the plexus.

During this plexus formation there is a steady extension toward the surface, and by the time anastomoses have formed between neighboring clusters sprouts have grown to the surface and started to extend in the region superficial to the plexus and also ventralwards. It now becomes possible to study with more minuteness the changes which are going on, since these sprouts are quite superficial and are developing in a plane parallel with the surface. Observation and successive records of these sprouts in the living chick reveal a rapid extension ventrally and also anteriorly, accompanied by a plexus formation. Two or three sprouts are seen to lead and soon numerous connections develop between them. Various portions of the irregular plexus thus formed enlarge and become more densely packed with blood which continues to back up from the vein. Then new sprouts grow out in advance and the same process of extension accompanied by plexus formation is repeated. If a single sprout is selected and frequent careful drawings are made the changes are seen to be rapid and striking. The sprout becomes wider and longer. Branches appear, and they in turn increase in width and length. From a branch a connection forms with the original sprout, thus forming a loop. New branches and connections are formed, making a plexus. Branches from neighboring loops or plexuses meet one another and anastomose. The several parts of the plexus are quite irregular in size. Most of the lymphatic vessels are several times as wide as a blood capillary, while some of the connections are as small as, or even smaller, than a blood capillary. Throughout the blood in these new-forming lymphatics is markedly darker in color than the circulating blood.

This work thus corrects the idea that lymphatics are transformed blood capillaries. They bud out from the veins as lymphatics and gradually invade the body as a new type of vessels.

Miller's work (97) on the anterior lymph sac is chiefly valuable in giving an excellent chance to contrast the method of reconstruction with the method of injection in an area where an injection can be obtained. Figure 14 is copied from Miller's fig. 6, and fig. 15 is from an injection by E. L. Clark's (27, fig. 2) of the plexus in the same stage. The points of the apparently isolated islands of lymphatics and the separation of the sac from the veins show by Miller are errors which are cleared up by E. L. Clark's work. The jugular lymphatic plexus is connected with the vein in a number of places and is a continuous endothelial-lined plexus of lymphatics.

The jugular lymph plexus develops into a small sac (Miller's fig. 6) which becomes a single jugular gland. This small, fusiform gland has been studied by a number of observers and has been worked out in all its relations by Jolly (59) in his interesting paper on the lymph glands in buds.

Miller (97, page 489) quotes Mierzewski as having seen rhythmical contractions of the jugular lymph sacs. A reference to Mierzewski's paper, however, will show that he consistently speaks of posterior lymph heart and jugular lymph plexus and his reference to pulsation (page 479) is a confirmation of Sala's observations in the posterior lymph hearts. Miller notes the absence of muscle and the point should be emphasized, because the jugular lymph sac in birds becomes not a lymph heart, but a lymph gland.

The iliac lymphatics in birds are very well brought out by Jolly (59) in his article on lymph glands. He does not consider their origin, but his fig. 15 shows their distribution extremely well, and his fig. 11, pl. XI, gives the exact histological picture of a lymph sac with connective tissue bridges for a bird on the 18th day of incubation. This is the method by which the primary sacs become lymph glands, as is shown in section VI.

6. PRIMARY LYMPHATIC SYSTEM IN AMPHIBIA, ANTERIOR AND POSTERIOR LYMPH HEARTS.

It is not possible at this time to give a complete account of the development of the lymphatic system of the amphibia and reptilia, but enough evidence is at hand to show that the lymphatics are derived from the veins. The remarkable subcutaneous lymph sacs in the frog have always attracted interest and stimulated a curiosity to unravel their development as was brought out by Langer (75) in 1868. Some recent experiments of Abel (1) which combine anatomical and pharmacological methods add to this interest.

Meltzer (95) had published experiments to show that a smaller dose of a drug produced convulsions when injected into a cardiectomized frog than into a normal one. He concluded that the drug traveled through the tissue spaces.

Abel found that a dye, acid fuchsin, when injected into the lymph sacs of a normal frog was distributed widely over the body by the blood vessels. In a cardiectomized frog a small dose injected into the

muscle (where there are no lymphatics, only tissue spaces) traveled exceedingly slowly; on the other hand, a small amount of the fluid injected into a lymph sac traveled rapidly, but in a zone limited exclusively to the lymph sacs, according to their anatomical connections. When the dye reached the lymph heart in the cardiectomized frog it was pumped, not through the heart to the systemic vessels, but through the vertebral veins to the nervous system. Thus is explained the marked effect of the small dose. If the lymph hearts were killed the convulsions did not take place.

It has been shown that the anterior lymph hearts of the amphibia bud off from the vertebral veins (Hoyer 49), or in a more primitive position from branches of the earlier segmental veins which are going to form the vertebrae (Knower 74). They develop earlier than the posterior hearts and have been found in *R. palustris*, *R. sylvatica* and *R. virescens* measuring about 6.5 mm., and in *R. temporaria* and *Bufo vulgaris* measuring about 4.5 mm. Hoyer noted the presence of blood in the early stages of both anterior and posterior hearts. Knower brings out the fact in connection with the anterior hearts that they lie on the myotomes from which they derive the striated muscle of their wall. This is, I think, a very important point. The particular myotome Knower has shown may vary.

The pulsations of the anterior lymph hearts can be readily seen in the dorsal surface of the tadpole, just behind the pronephros, and thus they can be injected.

From the anterior lymph heart develop two symmetrical vessels, one of which runs forward toward the head, the other backward toward the tail. The posterior duct as described by Hoyer divides into two branches, one running to the tip of the tail dorsal to the myotomes, the other passing back along the ventral margins of the myotomes to form the ventral caudal trunk. It is the branches of these two caudal trunks which have been studied so much in the living specimen. There is also a lateral branch which runs caudalward from the anterior lymph hearts on the lateral surface of the myotomes half way between the dorsal and ventral branches. This lateral trunk subsequently connects with the posterior lymph heart.

Wieliky (153), Jossifov (62-65), and Favaro (36) thought that the posterior lymph heart arose from the dilation of the caudal lymph trunks which grow from the anterior lymph hearts, and Jourdain (61) describes them as being formed by a rapid destruction of con-

nective tissue. Knowler and Hoyer, however, have found that they bud off from the posterior vertebral veins considerably later than the anterior lymph hearts, and they have been well worked out by Barański (9). Barański shows that they arise by several buds from the endothelium of the posterior vertebral vein and its branches. His fig. 1 shows particularly well the heaping up of the endothelium so that it looks like a solid mass at the point of origin. Practically nothing is known of the origin of the deep lymphatics in amphibia.

It has thus been shown that the lymph hearts of amphibia arise as buds or sacs from the endothelial wall of segmental veins. They lie on the myotomes, from which they derive the striated muscle of their wall.

The subcutaneous lymph sacs of the anura are secondary structures. This was found out by Ranvier (116) in 1896, and also by Knowler and Hoyer. They are derived from ducts which grow from the lymph hearts. They have been comparatively little studied. Hoyer (49) found that the large sac on the ventral surface of the head (sacc. submaxillaris) developed from a branch of a duct from the anterior lymph heart, and that the lateral sacs are derived from the lateral lymph trunks.

This point has been just shown in connection with the sacs of the extremities by Frl. Goldfinger (38), who has injected the primary ducts and then the increasingly denser lymphatic plexus up to the final lymph sacs. This process of cavernization could be studied here by silver nitrate injections.

Since the above was written Hoyer (49a) has published an article in which he gives a general review of the origin of the lymphatic system in vertebrates, and Hoyer and Udziela have given the first comprehensive description of the lymphatic system in a urodele (49b). In a salamander larva the lymphatic system opens into the veins first through symmetrical axillary lymph sacs or sinuses, and secondly through a series of subcutaneous lymph hearts which pump the lymph into the vena lateralis (Meyer 88a and Marcus 87a).

There are six longitudinal lymph trunks, four superficial and two deep (Hoyer 49b, Taf. XII, Figs. 1, 2 and 5). (1) The median, dorsal, longitudinal lymph trunk, which extends from the point of the tail to the middle of the head; (2) a medial, ventral caudal trunk, which branches around the cloaca and opens into the inguinal sinus as is shown in fig. 2 (49b); (3 and 4) symmetrical lateral trunks, which

lie external to the myotomes and open in part into the segmental lymph hearts and in part into the axillary sinus, and (5 and 6) the deep subvertebral lymph trunks. The subvertebral lymph trunks are paired vessels with many anastomoses which extend from the tip of the tail to a point opposite the stomach, where they unite to form a cisterna chyli. From the cisterna chyli four vessels run forward to the axillary plexus, the paired thoracic duct and the two paravertebral lymph trunks.

The axillary lymph sacs open by three or four branches into the cardinal veins and receive the following vessels: (1) The lateral superficial lymph trunks; (2) the paravertebral vessels; (3) the thoracic duct; (4) vessels of the cranial part of the stomach and esophagus; (5) a vessel which runs in the groove between the pericardial and peritoneal sacs; (6) a vessel which comes from the base of the skull; (7) superficial vessels from the side of the head, and (8) the lymph vessels of the forelegs.

Besides the axillary sac there are two other sacs or sinuses, one of which lies dorsal to the aorta at the base of the heart, namely, the sinus lymphaticus cordis (Hoyer 49b, fig. 5), and is in the course of the jugular lymph trunks and the other of which makes the paired sinus of the inguinal region.

The segmental lymph hearts receive three groups of vessels: (1) branches from the subvertebral lymph vessels (which agrees with the findings of Marcus 87a, in *Gymnophionem*; (2) dorsal and ventral segmental branches, and (3) the longitudinal lymph trunk. They open into the vena lateralis. The segmental lymph hearts and the sinus lymphaticus cordis have striated muscle in their walls. It is clear that a knowledge of the origin of this system would be of great value, as Hoyer says: "Erst wenn die berührten noch zweifelhaften Punkte durch weitere Untersuchungen vervollständigt sein werden, wird eine gewisse Grundlage zu vergleichenden Betrachtungen des Lymphgefäßsystems der niederen Wirbeltiere geschaffen sein" (49b, p. 555).

Stromsen (145) and Huntington (57) have worked on the development of the lymphatic system in reptiles. Stromsen shows that the posterior lymph hearts develop in relation to the coccygeal veins. He describes the process as a combination of veins and dilated spaces similar to Sala's description for the corresponding hearts in birds. This method of formation has now been disproved by the observation

of the process in the living chick. He notes that the striated muscle of the heart comes from the myotomes. Huntington (57) states that in the reptiles there are jugular lymph sacs like those of mammals. The lymph vessels of reptiles he describes as arising from tissue spaces.

V. PERIPHERAL LYMPHATIC SYSTEM.

1. IN THE PIG.

A. FROM THE JUGULAR LYMPH SACS.

I shall again base the description of the peripheral lymphatics on the vessels in the pig, since more of the main trunks have been worked out in that form than in any other. The description is taken from the work of A. H. Clark (24), of Heuer (43) and myself.

The development of the jugular sac in the pig has already been given in section IV. A study of the distribution of the lymphatic vessels from the jugular sac must be based on the form of the jugular sac as seen in figs. 7 and 8. In fig. 7 there is a primary sac between the internal and external jugular veins and a complete arch of lymphatic capillaries extending into the dorsal lymphatic area. This entire plexus becomes transformed into the sac, so that, as shown in fig. 8, the sac consists of a stem along the internal jugular vein and a complete dorsal arch. Through following the fate of this sac A. H. Clark divides it into three parts, the sac stalk; the anterior curvature along the internal jugular vein; and the apex, in the posterior triangle of the neck.

The sac stalk becomes the deep jugular lymphatic trunk. It is usually one large vessel, but may give off lymphatics which run directly to the pharynx. The anterior curvature becomes a lymph gland and it sends vessels to the pharynx and Eustachian tube and to the nose.

The apex of the sac, as is seen in fig. 8, becomes the largest part of the jugular sac. This part lies in the posterior triangle of the neck and develops in the pig into one gland, the largest in the neck (Clark 24). This corresponds to the group of glands in the posterior triangle of the neck particularly well shown in Bartels (10) fig. 30 from new born child.

The fact that the jugular lymph sac in the pig develops into two lymph glands and the deep jugular lymphatic trunk is well brought

out in fig. 16 from a pig 5.5 cm. long. The sac is shown in the depth and the swollen anterior curvature (deep jugular gland) and apex (gland of the posterior triangle) stand out through the relatively small size of the connecting vessel.

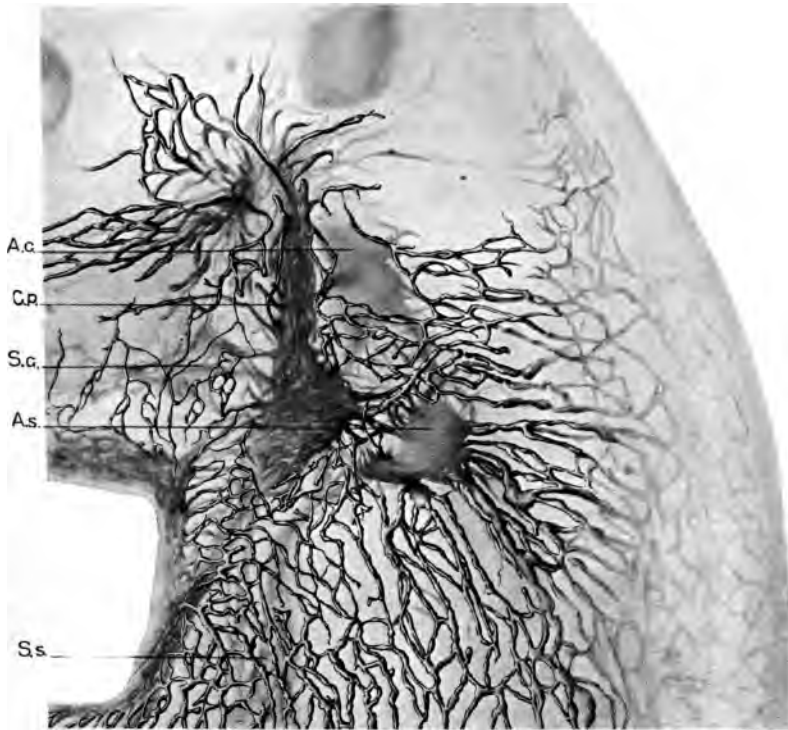


FIG. 16.—Injection of the jugular lymph sac, the superficial cervical plexus, and the superficial lymphatic vessels in the neck of a foetal pig 5.5 cm. long. After A. H. Clark (24). The specimen was fixed in formalin 10 per cent formol and made transparent by the Spalteholz method. Magnified 7 times. The figure is to be compared with fig. 5 in the Amer. Journ. of Anat., 1904, Vol. 3, p. 118, which shows a complete injection of the superficial lymphatics of the same stage. A. s. = apex of the lymph sac which forms the lymph gland of the posterior triangle of the neck; A. c. = anterior curvature of the lymph sac, which forms the deep jugulo-pharyngeal lymph gland; C. p. = superficial cervical lymphatic plexus; S. g. = lymphoglandular submaxillaris; S. s. = stalk of the jugular lymph sac.

From the apex of the sac superficial lymphatic vessels grow both from the dorsal and from the ventral border. Those from the dorsal and caudal border make a large plexus of superficial lymphatics which,

as shown in fig. 8, grow (1) over the scapular region and (2) over the occipital region. The lymphatics from the ventral border of the apex are most interesting and complex. They grow ventralward as shown in figs. 8, 16 and 17 and form a very extensive plexus along the course of the external jugular vein, lateral to the sternocleidomastoid muscle. This is the superficial cervical plexus and it becomes a large group of superficial lymphatic glands in the adult pig (Clark 24).

The cervical plexus not only connects with the apex of the sac, but, as is shown faintly in fig. 16, its caudal end has a group of vessels that connects with vessels that follow the external jugular vein. In a mesial sagittal section of injected pigs 5 to 6 cm. long it is plain that a very abundant plexus of lymphatics from the root of the sac stalk completely surrounds the external jugular vein, and this external jugular plexus has numerous connections with the cervical plexus at its caudal end. The plexus around the external jugular vein empties mainly into the sac stalk, but a single vessel may join the external jugular vein. It must be said in general that the cervical plexus develops from the jugular sac in two places: (1) from the apex of the sac over the sternocleidomastoid muscle, and (2) from the sac stalk along the external jugular vein.

From the cervical plexus there are seven sets of lymphatic capillaries (fig. 16). From the cerebral end of the cervical plexus vessels grow (1) behind the ear, and into a new facial plexus from which vessels grow (2) between the eye and the ear, and (3) over the face. From the ventral border of the cervical plexus there is (4) a set of submaxillary lymphatics which supplies the lower jaw and tongue and (5) superficial lymphatics for the skin of the neck. From the caudal end of the cervical plexus grow (6) the superficial lymphatics of the arm, and (7) the superficial lymphatics of the thorax.

Thus to sum up the superficial lymphatics for the jugular sacs: from the sac stalk and anterior curvature arise the pharyngeal, Eustachian and nasal vessels, from the apex of the sac arise the suprascapular and occipital lymphatics, while from the cervical plexus arise the posterior auricular, temporal, facial, submaxillary, anterior cervical and the superficial lymphatics of the arm and thorax. These groups of lymphatics begin as distinct plexuses of vessels which arise from the sac or from the secondary cervical plexus which in turn comes from the sac. In fig. 8 is shown particularly well how distinct the groups may be at the start. Subsequently all of the groups anastomose and this is an important point.

In fig. 8 it will be seen, for example, that the large vessels of the suprascapular region drain into the apex of the sac, but some of them have small connections with the cervical plexus. In fig. 16 these connections are so much more abundant that they exceed in number



FIG. 17.—Injection of the jugular lymph sac and the cervical lymphatic plexus of a fetal pig 7.5 cm. long to show the relation of the developing lymph glands to the jugular sac. After A. H. Clark (24). Magnified 5 times. Fixed in 10 per cent formol and cleared by the Spalteholz method. A. s. = apex of the sac, which is now a lymph gland in the posterior triangle of the neck; the anterior curvature of the sac, which is now a deep jugular lymph gland, lies behind the sternocleidomastoid muscle. C. p. = superficial cervical lymphatic plexus, which will become a group of lymph glands. At the cerebral end of the cervical plexus a developing facial lymph gland; S. g. = lympho-glandula submaxillaris.

those that pass to the sac itself. This point of the anastomoses of the difficult groups of vessels is well shown in fig. 5 (Sabin 130), where practically all of the superficial lymphatics in the embryo pig 5.5 cm.

long have been injected from one puncture. This is a complete injection, which it is clear that fig. 16 is not. In fig. 5 (Sabin 130) for example the lymphatics between the eye and ear are terminal ducts.

Good examples of the anastomoses of different groups of lymphatics are shown in fig. 16 in the vessel which connects the submaxillary and the facial plexus; moreover the thoracic, cervical and brachial plexuses are continuous.

In the embryo pig from 5 to 5.5 cm. long there are no valves in the ducts. It is also the stage of the simple primary plexus of lymphatics. In fig. 16 a secondary plexus of finer lymphatics is growing more superficially and by the time the embryo is from 6 to 7 cm. long there is a deep plexus with valves and a finer-meshed superficial plexus.

The relation of the jugular lymph sacs to lymph glands is shown in fig. 17. Here it is clear that the sac stalk and anterior curvature lie beneath the sternocleidomastoid muscle and that the apex of the sac lies in the posterior triangle of the neck. That the cervical plexus is now a lymph gland is clear, and there is a facial and submaxillary gland also. The cervical plexus becomes a group of glands.

The account of the peripheral lymphatics from the jugular sacs is not complete without mention of the lymphatics of the heart and lungs. These lymphatics have not yet been worked out, but it can be said that there are two sets of lymphatics for the lungs, the deep and the superficial or pleural. The deep lymphatics develop from the jugular sacs; they follow the trachea and are present in a pig measuring 23 mm. and a human embryo measuring 20 mm. I have not injected them and when reconstructed they show the Mayer-Lewis Anlagen; that is, in reconstruction they split into a chain of beads. The pleural lymphatics I have injected from the retroperitoneal sac, through the diaphragm to the caudal surface of the lung. Injections of the thoracic duct as, for example, fig. 7 (Nuck 100), also shows vessels from the thoracic duct to the lung. Therefore the lung seems to have a double supply of lymphatics, part from the jugular sac and part from the renal sacs by way of the diaphragm and by way of the thoracic duct.

B. THE PERIPHERAL LYMPHATICS FROM THE RENAL SACS.

The retroperitoneal sacs give rise to the lymphatics of the abdominal viscera except the kidneys. The sac as it lies in the root of the mesentery has been figured by Heuer (43). It spreads over the ventral

surface of the Wolffian bodies and supplies their capsule as well as the reproductive glands. From the sac vessels grow to the stomach and the entire intestine, including the rectum. The lymphatics of the diaphragm can be injected from the retroperitoneal sac. The development of the lymphatics of the diaphragm, liver, capsule of the spleen, adrenal and pancreas has not yet been worked out.

Heuer (43) has followed the lymphatics of the intestine from the retroperitoneal sac to the ultimate lacteals. While the embryo is growing from 3 to 4 cm. long a very abundant plexus of capillaries spreads out in the mesentery. The lymphatics reach the wall of the intestine by the time the embryo is 4 cm. long. They first enter the submucosa and form then a primary plexus. Heuer's figures 9 and 10 show that the early vessels in the submucosa have a segmental arrangement, which is, however, nearly lost as the complete plexus forms. The plexus of the mucosa, and of the serosa are both secondary. The lacteals develop from the mucosal plexus and are present in a pig measuring 9 cm. The mesenteric vessels and the submucosal plexus develop valves.

The iliac lymphatics and the thoracic duct: The lymphatics which arise from the mesonephritic veins on the two sides form a very simple pattern. Starting from the veins at the hilus of the two Wolffian bodies they grow caudalward along the edge of the Wolffian bodies to make the iliac sacs, and cerebralward along the dorsal surface of the aorta to form the cisterna chyli. The lymphatics dorsal to the aorta grow in two directions: (1) caudalward to form a chain of prevertebral lymph nodes and (2) cerebralward to form the thoracic duct.

The iliac lymphatic sacs are two long symmetrical sacs extending from the hilus of the Wolffian bodies to the level of the bifurcation of the aorta. In an embryo 23 mm. long they drain into the mesonephritic veins. By the time the embryo is 25 to 27 mm. long this connection is lost and the two iliac sacs converge into the median cisterna chyli dorsal to the aorta.

The caudal end of the iliac lymphatics in the pig is a sac of considerable size, from which three sets of lymphatics can be injected: the ilio-lumbar, the femoral and a plexus which surround the umbilical arteries. The ilio-lumbar superficial lymphatics are very conspicuous in the pig, as shown in figs. 4 and 5 (Sabin 130). This is in marked contrast to the human embryo, where they form part of the inguinal group.

2. PERIPHERAL LYMPHATICS IN OTHER FORMS.

The pattern of the superficial lymphatics in the human embryo is shown in the two figs., 505 and 506 (Sabin 134). This was a remarkable specimen of an embryo 5.5 cm. long which had been kept in formalin some time. On transferring it to freshly made up alcohol, air filled the entire superficial lymphatic system. It shows the primary superficial lymphatic plexus. Valves in a few ducts, namely, the occipital, the thoracic and the inguinal, indicate the oldest vessels. The differences between the superficial pattern in the human and in the pig are mainly the fact that in the human embryo the thoracic lymphatics are much more prominent, draining into the axilla, while in the pig they drain into the superficial cervical plexus, and all the posterior lymphatics drain into the inguinal region in the human embryo. In the human embryo the deep lymphatics for the arm grow from an extension of the jugular sac along the primitive ulnar veins, and the deep lymphatics for the legs grow from the iliac sac along the femoral veins. This shows in fig. 493 (Sabin 134).

Rabbit and cat embryos are like the human in having a marked development of the jugular sac along the primitive ulnar vein (Lewis 76). In this respect they differ from the pig where the axillary lymphatics are smaller. In fig. 7 is shown a deep vessel from the jugular sac, which is, I think, the axillary duct in the pig. In the rabbit and the cat the axillary sac may possibly arise independently of the jugular sac, and in the rabbit a conspicuous chain of peripheral vessels grows along the thoraco-epigastric vein (fig. 8, Lewis 76). Injections of cat embryos show that the axillary lymphatic trunk is very large.

The superficial pattern of the lymphatics in bovine embryos, as shown by Polinski (107), is much like that of the pig.

In the chick the primary lymphatic vessels in the skin connect the posterior lymph heart with the jugular lymph sac along the lateral line in the course of the thoraco-epigastric vein Mierzewski (96) and E. L. Clark (27 and 29).

VI. ORIGIN AND DEVELOPMENT OF LYMPHATIC GLANDS AND THEIR RELATION TO PRIMARY LYMPH SACS.

It is easy to prove that the lymphatic sacs in mammals and the jugular lymph sac in birds become in part lymph glands. I shall define primary lymph glands as those which are derived from the primary lymph sacs.

From the jugular lymph sac in the pig it is clear in fig. 16 that two lymph glands develop: (1) the deep jugular lymph node which drains the pharynx and (2) the node in the posterior triangle of the neck which drains the skin of the anterior part of the body. A. H. Clark (24) has shown that these two glands are single glands in the neck of the adult pig and that they become the largest of all the cervical glands. In the human embryo these two glands are represented by groups of glands, the glands of the posterior triangle and the deep jugular glands. In the human embryo also the group of deep axillary glands comes from an extension of the jugular sac along the primitive ulnar vein and hence they are primary lymph glands. Thus the primary nodes for the anterior part of the body are (1) deep jugular nodes, (2) the nodes of the posterior triangle, (3) the axillary nodes. The relation of the primary lymph gland, both to the various structures of the neck and to the stalk of the jugular sac, that is, to the jugular lymph trunks, is shown especially well in fig. 17 from pig 7.5 cm. long. The anterior curvature and the sac stalk lie behind, that is, medial to the sternocleidomastoid muscle. Of the primary nodes in the neck the deep jugular nodes drain a restricted area, namely, the pharynx and nose; the axillary lymph nodes drain the arm, and the large node of the posterior triangle drains all the rest of the head, face, neck and thorax, either directly or through the superficial cervical plexus. The nodes of the superficial cervical plexus are secondary to the primary lymph sac nodes. It has been brought out that these groups of lymphatics do not remain distinct as they arise, but all become connected with each other through anastomoses of the lymph vessels.

From the retroperitoneal sac develop the retroperitoneal lymph nodes. From the iliac sacs in the pig there is a chain of small nodes lateral to the aorta and a large group of glands on either side opposite the bifurcation of the aorta. These are primary iliac nodes. Dorsal to the aorta is a chain of nodes from the lower end of the cisterna chyli to the bifurcation of the aorta. Thus the renal lymphatics give rise to three groups of primary lymphatic glands: (1) præ-aortic or retroperitoneal, (2) symmetrical iliac nodes lateral to the aorta, and (3) præ-vertebral nodes dorsal to the aorta.

The secondary lymphatic nodes develop along the lymphatic vessels. The most extensive group of secondary nodes in the embryo pig is the one which comes from the superficial cervical plexus along the external

jugular vein. In fig. 17 this plexus is shown as one large lymph gland. From the cerebral end of the plexus vessels are forming a gland at the point where the lymphatics divide to form the facial and the temporal lymphatics. The cervical plexus in the adult pig becomes a group of at least a dozen small lymph glands. In both figs. 17 and 18 there is a developing submaxillary lymph gland along the course of the facial branch of the linguo-facial vein (fig. 8). The small facial node and the submaxillary node are tertiary nodes compared with the jugular lymph sac. They show how lymph glands develop at points where lymph ducts radiate out on their development. The primary, secondary and tertiary nodes are constant in the pig.

The mesenteric glands are secondary for the retroperitoneal sac. The secondary glands from the iliac sac are very simple, for there are only two of them: (1) The ileo-inguinal gland, which is very characteristic of the pig and which remains as a single gland. Its position is readily made out in fig. 4 (Sabin 130), for the superficial lymphatics of the posterior body wall radiate to it. Like the superficial cervical glands it is superficial. (2) The inguinal glands in the pig become a large group of superficial glands, the position of which can be made out in fig. 5 (Sabin 130). The lymph ducts from the inguinal lymph glands develop to the leg, the ventral abdominal wall and the external genital organs. The efferent vessels of the inguinal group of glands and ileo-inguinal gland converge to the large group of primary iliac glands opposite the bifurcation of the aorta.

The histological development of lymph glands is now well understood. The work of Saxer (138) on developing lymph glands considers primarily the development of lymphocytes which I shall not attempt to discuss in this paper, confining the work to the development of the gland as a whole. From the time of Breschet (16) it has been known that lymph glands begin as a plexus of lymphatic ducts. Their development can be followed through the work of Ranvier (117), Bartels (10), Gulland (39), Kling (72), Jolly (59), Lewis (77), and Sabin (131).

The primary lymph glands, which, with the exception of the post-aortic, develop out of sacs, begin by a bridging of the sac by bands of connective tissue covered by endothelium. In the case of the retroperitoneal sac these bands are never absent (figs. 498 and 499, Sabin 134). For the jugular lymph sac they show well in human embryo 30 mm. long in fig. 495 (Sabin 134). That these bridges are entirely covered

by endothelium is best brought out by total mounts of silver nitrate injections. This has been done for the retroperitoneal sac in the pig. When the primary sacs are thus completely bridged by these bands they are practically a dense plexus of lymphatic capillaries and are therefore in the first stage of the development of lymph glands. At this stage the connective tissue septa are undifferentiated and contain only mesenchyme and blood capillaries.

We are now in a position to consider the question of which are the first lymph nodes in the embryo. This point has been discussed by Lewis (77) and Sabin (131 and 134). It depends on the criterion used. If the criterion is recognizing the formation of a plexus of lymphatic capillaries with undifferentiated connective tissue bridges as the anlagen of a gland, then it is clear that the primary lymph glands, that is, those that come from the lymph sacs, are the first lymph glands to begin for each region of the body. Thus the jugular-subclavian sac in the human embryo is extensively bridged at 30 mm. and is therefore a plexus of lymphatics, the anlage of a lymph gland. At this stage we are agreed there are no lymphocytes (Kling (72), Lewis (77) and Sabin (131)). If, on the other hand, the criterion is the development of the first lymphocytes in the body, we must say that this point has not been reinvestigated since all the primary lymph nodes of the embryo have been determined. Saxer (138, p. 381) notes that lymph nodes are beginning in the neck, the posterior mediastinum, the retroperitoneal tissue, the outside of both hips and along the Wolffian bodies in bovine embryos 4.5 cm. long, and in sheep embryos of the same length describes undoubted wandering cells in the same areas. Kling (72) has no specimens of human embryo between 31 mm., where, he says, there are no lymphocytes in the axillary glands, and 70 m., when lymphocytes are present in many glands. Lewis (77) finds lymphocytes in human embryos 42 mm. long. He finds that they occur practically simultaneously in the glands around the internal jugular vein (primary lymph nodes) and certain "isolated subcutaneous lymph glands," of which he figures one or two along the linguo-facial vein and its branches. The relation of these glands to lymphatic vessels will be readily made out by a comparison of fig. 8 and fig. 16. Figure 8 shows the ducts from the sac along the linguo-facial vein at a stage even younger than Lewis's isolated glands, namely, a pig measuring 3.5 cm. Moreover the beginning cervical plexus from which these submaxillary vessels come

shows plainly in fig. 7 from an embryo much smaller, namely, one measuring 18 mm., and they are readily injected in embryos 30 mm. long, fig. 2 (Sabin 130). Thus it is almost certain that the ducts for Lewis's early facial glands in human embryos were present, but could not be found, in sections. It is probable that the lymphocytes begin in human embryos which measure about 40 mm. and they probably develop almost simultaneously in the primary and in the secondary glands.

The plexus stage of developing lymph glands has been shown by reconstruction by Kling (72) and Lewis (77), and by injection in fig. 10 (Sabin 131).

The whole question of the structure of a lymph gland may be summed up in a word by a quotation from Ranvier (p. 1038 in 117): "Un ganglion lymphatique est un angiome caverneux lymphatique qui a été d'abord angiome simple." The development of a gland involves, however, two processes: (1) the formation of lymph sinuses out of lymph plexuses, and (2) the formation of lymph cords and lymph follicles in the trabeculæ. A comparison of Jolly's (59) plates from lymph glands in birds, Kling's (72) figures of human lymph glands, and my own (131) from the developing glands in the pig, will show that the relative proportion of these two parts varies exceedingly in different glands.

The formation of lymph sinuses can be best understood by beginning with Jolly's figures. He shows that in the birds, instead of the mammalian sinus, there is a diffuse plexus of lymphatic vessels with lymphocytes in the septa between the vessels. He also brings out the fact that very large vessels may pass directly through the gland. Thus it is clear that the sinus of birds is a simpler structure than that of mammals. On this account the complete covering of endothelium is very plain in Jolly's figures. Out of a plexus of capillaries such as Jolly shows, the mammalian lymph sinus is formed, as can be seen in fig. 15 (Sabin 131), by an increase in the density of the lymphatic plexus until the septa are reduced to a framework of reticulum covered by endothelium. In this figure all the stages of the formation of a sinus can be seen in a single section. In the lower part of the specimen every lymphatic vessel is plain with its complete lining of endothelium, while in the upper part the septa are in places already reduced to a line. This stage is from an embryo pig 24.5 cm. long, which is the

best stage for studying the formation of the sinuses. The question of the development of reticulum has been taken up by Mall (84).

In regard to the formation of the lymph cords and follicles, the fundamental point is the question of the origin of the lymphocytes themselves. This, however, I shall not undertake to discuss. The lymphocytes may occur diffusely in all of the trabeculæ of the developing node and this is the simplest form of a gland. This form is shown by Jolly (59) in his plate IX, fig. 3, for the lumbar glands in birds, by Kling (72), plate XXVII, fig. 11, for the axillary glands in human embryos and in fig. 9 (Sabin 131) from the primary jugular lymph glands in the pig. It will be noted that all of these figures are from primary lymph glands. The definite follicles may develop in the center or in the periphery of a node, and there is every possible variation in the proportion of the diffuse cords and the definite follicles. There are two processes in the development of the follicle: (1) an increase in the number of lymphocytes forming a definite clump, and (2) the formation of a tuft of blood capillaries. Both the cords and the follicles form along the blood vessels, the follicles coming at the capillary bed.

As I have said, the early glands which come from the primary sacs pass through the form of diffuse distribution of the lymphocytes. Follicles which develop later, however, may begin at once as clumps of lymphocytes, making very definite follicles surrounded by a lymphatic plexus. A number of such tiny follicles are to be seen in fig. 16 (Sabin 131), which is the developing group of inguinal lymph glands in a pig 24.5 cm. long.

Lymph glands may be either simple, consisting of one follicle with a peripheral sinus, or compound, with many follicles and cords, and both peripheral and central sinuses.

The subject of hæmolymph glands has been taken up by Helly in the *Ergebnisse für Anatomie und Entwicklungsgeschichte* for 1902.

VII. COMPARATIVE MORPHOLOGY OF THE PRIMARY LYMPH SACS, LYMPH HEARTS, AMPHIBIAN LYMPH SACS AND LYMPH GLANDS.

We are now in a position to consider the comparative morphology of lymph sacs and lymph hearts. I shall not attempt to analyze the work of Favaro (36a) and of Allen (2-4) on lymphatics in fishes,

both of whom state that vessels may function now as veins and now as phatics, but beginning with the amphibia, it is certain that lymphatics bud off from the veins in certain definite areas and form plexuses which are transformed into sacs. The essential structure of these sacs is the endothelial lining. A primary lymph sac is therefore one which buds off from the endothelium of a vein.

In the amphibia, probably in the reptilia, and in the case of the posterior lymph sacs in birds, the primary sacs bud off from segmental veins and rest on the myotomes. They derive striated muscle from the myotomes, which has been shown at least in the case of the anterior lymph hearts in amphibia (Knower 74), and the posterior lymph hearts in reptiles (Stromsen 145) and pulsate rhythmically; in a word, they become lymph hearts.

The lymphatic sacs for the anterior part of the body in birds and all of the lymphatics in mammals do not come from the vertebral and coccygeal veins, but from the anterior cardinal veins in the neck, and the renal veins in the abdomen. These sacs do not lie on the myotomes, they do not receive striated muscle, but rather are transformed into lymph glands. Thus the bird represents a transition stage having a posterior lymph heart and a jugular lymph gland. I shall keep, therefore, the term anterior and posterior lymph hearts, and use the terms jugular and renal lymph sacs. The embryological classification is:

- | | | | |
|---------------------------|---|---|-----------------|
| Primary
Lymph
Sacs. | } | 1. Lymph hearts. | |
| | | a. Anterior, amphibia. | |
| | | b. Posterior, amphibia, reptilia and birds. | |
| | | 2. Lymph sacs (glands). | |
| | | a. Jugular—birds and mammals. | |
| | | b. Renal { Retroperitoneal. | |
| | | | Iliac. |
| | | | Cisterna chyll. |

The subcutaneous and deep lymph sacs of the amphibia are not primary, but secondary. They are transformed lymphatic ducts. Thus the amphibia have primary lymph sacs which become lymph hearts, lymph ducts which become secondary sacs, and lymphatic capillaries. Mammals have primary lymph sacs which become primary lymph glands, lymphatic ducts which develop valves and along which secondary glands are formed, and thirdly, lymphatic capillaries without valves.

VIII. VARIOUS OTHER THEORIES IN REGARD TO THE ORIGIN AND DEVELOPMENT OF THE LYMPHATIC SYSTEM.

1. MAYER-LEWIS ANLAGEN.

In the preceding pages has been given a consistent account of how the lymphatic system buds off from the veins and gradually invades the body. The places where the lymphatics bud off vary in different forms; in the amphibia and reptiles, and in the case of the posterior lymph hearts in birds the primary lymph sacs lie in the myotomes and receive striated muscle and become lymph hearts. In the higher forms the anterior lymphatics come from the anterior cardinal vein and its branches, the caudal lymphatics do not develop (mammals) and the

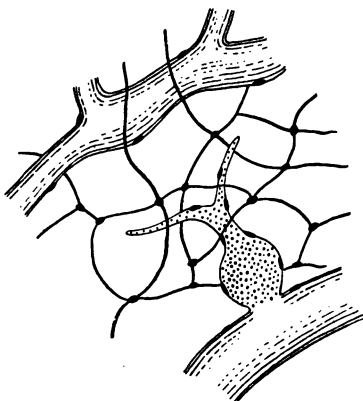


FIG. 18.—Diagram to show the theory of Ranvier and myself that the lymphatics arise from the veins. The veins are striped, the lymphatics dotted.

posterior lymphatics come from the vena cava and renal veins. From these points of origin can be traced all the lymphatics of the body down to the ultimate capillaries.

This theory has not been developed without opposition. In section I it was made clear that the first theory of the origin of the lymphatics was that they arose in the periphery as dilated tissue spaces and grew towards the center, Gulland (39). This was questioned by Sala (137), showing that the first lymphatics in birds were against the veins, and by my proving in 1902 that the jugular lymphatics are the first lymphatics in mammals and that they bud off from the anterior cardinal veins. Thus I substituted for the theories shown in

figs. 1, 2 and 3 the simple hypothesis of Ranvier, shown in fig. 18. This indicates that lymphatics bud off as lymphatics from the veins and have the same relation to tissue spaces as have blood capillaries.

The most difficult obstacle in regard to this theory is what we call Mayer-Lewis anlagen. Mayer (89) noted in the tadpole's tail certain isolated vessels which he thought were evidences of the degeneration of blood vessels.

Ranvier (114, p. 578) in studying the plexus of lymphatics of the great omentum in a new-born cat noted that a great many of the vessels terminated in cul de sacs. Some were attached by extremely slender threads, so that they looked as if they were becoming isolated by the atrophy of the intermediate part. Since in the adult cat there are no lymphatics in the omentum, Ranvier interpreted this as a method of degeneration of lymphatic vessels.

The study of the method of degeneration of blood and lymphatic capillaries is an important one. It is obvious that there must be a destruction of capillaries, since a single vessel or a few vessels come from a plexus of capillaries. In Clark's observations (25, p. 191) the usual method of degeneration is by retraction of the processes, but he has also observed (26, p. 410) together with Mayer and Ranvier, that an occasional segment of a blood capillary or lymph capillary may become detached and be left to atrophy. This is in accord with some of the findings of pathology. We must, therefore, conclude that detached, degenerating vessels do occur, though they do not represent the usual method of degeneration.

F. T. Lewis (76) found that in reconstructions lymphatics looked like chains of beads, and to explain this phenomena he suggested that lymphatics might grow by small detached portions of blood vessels. These hypothetical lymphatic anlagen we call Lewis anlagen.

It is clear in watching lymphatics grow, that they never pick up isolated vessels, but grow by the increase in the protoplasm of their own wall; yet it is clear also that reconstructions of uninjected lymphatics appear like rows of beads.

To study this point it was necessary to submit the method of reconstruction to comparative tests with the method of injection and when possible with the method of growth in the living form. This has been done three times (Clark, E. R. 26; Clark, E. L. 27, and Sabin 135).

The best opportunity for studying this point is the tadpole's tail, where, in the living specimen, or in the tail fixed in alcohol, the entire

lymphatic system can be seen and drawn. This was done by Clark. The specimen was then cut in serial sections and both blood capillaries and lymphatics were reconstructed. Two reconstructions were made, one with the 4 mm. Zeiss objective, and the other with a 2 mm. Zeiss oil immersion lens. Both reconstructions show that neither blood capillaries nor lymphatic capillaries can be reconstructed completely. More is obtained with an oil immersion lens, but both powers show capillaries in the form of rows of beads (figs. 5, 6 and 7, Clark 26, copied as figs. 513 and 514, Sabin 134).

This test of Clark's is the best possible test, because it is a reconstruction of exactly the same specimen from which the original drawing was made. This point cannot be made in testing the method of injection and that of reconstruction. For this test I (135) used, however, symmetrical plexuses in the same embryo. An embryo pig 27 mm. was chosen in which there was an almost complete injection of the occipital superficial lymphatics. Many of the sprouts on the margin had been ruptured. The injected plexus could of course be reconstructed, while a reconstruction of the empty lymphatics on the opposite side showed the entire plexus split up into isolated vessels (figs. 6, 7 and 8, Sabin 135). On the injected side there was just one lymphatic vessel which did not receive the injection mass, and there was an extravasation just at its base. This is readily explained by the fact that vessels are often connected by very slender strands to the main plexus, as, for example, the vessel near the point of injection in fig. 18, and a rupture might readily occur in such an area before the end of the vessel was reached.

The test of the two methods has now been made a third time by Mrs. E. R. Clark (27) in her injection of the jugular lymphatic plexus in a stage corresponding to the one which Miller (97) has reconstructed. The two results are shown in figs. 14 and 15.

A comparison of the injected jugular sac in a pig 18 mm., shown in fig. 7, with reconstructions of the corresponding stages in the cat (figs. 60 to 62, Huntington 55), also brings out the same point that, wherever the injection method can be applied, it demonstrates more continuous lymphatics in an area than can be reconstructed, even as isolated vessels.

Undoubtedly an occasional blood capillary or lymphatic capillary may separate from the main plexus and atrophy, but the apparently isolated vessels found in serial sections along the course of growing lymphatics connect in life. Lymphatics do not grow by de-

tached blood vessels, and hence the hypothetical Mayer-Lewis anlagen do not exist, that is to say, they are not anlagen of lymphatics, but are parts of a continuous vessel. It is clear that reconstructions of growing lymphatics are valuable, even though they show the lymphatics as rows of beads, provided only the true lymphatics are included; that is to say, the endothelial-lined vessels. For example, Lewis (76, figs. 7 and 8) shows that the early lymphatics in the rabbit grow along the thoraco-epigastric vein.

2. EXTRA-INTIMAL AND PERINEURAL SPACES AND FENESTRATION.

Huntington and McClure (51) began their work on the lymphatic system in 1907 with a study of the jugular lymphatics in the cat. They thought that lymphatics began as clefts between the intima, the vein and the surrounding mesenchyme, so that for a time the wall of a lymphatic was half venous endothelium and half mesenchyme. These spaces they called "extra-intimal" lymphatics. As far as the jugular lymphatic sacs were concerned they subsequently abandoned this idea, accepting the theory that the jugular sacs are derived from the veins (Huntington and McClure 54), but they have since revived the extra-intimal theory in connection with the thoracic duct (Huntington 58).

Their work on the jugular sacs has been given in section IV. In general they accept the theory of the venous origin of the jugular sacs, but they confuse the picture (1) by the inclusion of some perineural spaces (fig. 22, Huntington and McClure 54) and (2) by the idea of fenestration.

The perineural spaces are an interesting phenomenon. I considered them in my first paper in 1902. Along the entire central nervous system and following the peripheral nerves are dilated tissue spaces. Within the spinal canal these spaces are the anlage of the spaces of the pia-arachnoid. Along the nerves we call them perineural spaces. All sections of embryonic tissue show them. They are of undoubted significance for the physiology of the growing nerves, but they never bear any relation to the lymphatics. Injections of them outline the nerves and never enter lymphatic vessels—occasionally they can be injected from the pia-arachnoid, but usually the dense tissue between the vertebræ prevents this.

The question of fenestration was considered in section IV. Parallel veins are formed by the same process of sprouting of endothelium

by which all other vessels are formed, and the term fenestration, giving the vague suggestion of the splitting of the wall of a vein once formed, does not describe the actual process and is misleading. We now know that the jugular sacs bud directly from the anterior cardinal vein as lymphatics and grow by the sprouting of their endothelium.

As has been said, Huntington and McClure believe that the jugular lymphatics come from the veins, but that they remain only as a means of communication between the veins and the lymphatic ducts. The lymphatic ducts they think develop in a variety of ways.

The entire question of the method of growth of the lymphatic system is now concentrated on a study of the methods of growth of the thoracic duct. It is probable that the thoracic duct arises in two places; that it is formed in mammals by an asymmetrical down-growth from the left jugular sac and by a plexus which arises symmetrically from the renal veins and grows along the course of the aorta. This second portion arises later than the first and is in the blood-filled stage (pig 23 mm.) when the jugular stalk is empty. These two anlagen grow as do all other lymphatics and join by the same process by which any lymphatic plexus is formed.

The opposing views are brought out in two papers, one by Huntington (58) and one by Kampmeier (66). Huntington describes a complicated method of origin of the thoracic duct, including (1) a jugular stalk; (2) extra-intimal spaces, and (3) general tissue spaces. Kampmeier, on the other hand, gives a clear and excellent presentation of the theory of the origin of lymphatics by the addition of tissue spaces.

I will begin with the work of Huntington (58). His memoir on the peripheral lymphatics has excellent photographs of sections, so that it is easy to see what he is considering as lymphatics. For example, figs. 1 to 9 are tissue spaces, figs. 10 to 12 are extra-intimal spaces, and figs. 13 to 19 are probably lymphatics. Figure 25 shows the iliac sac labeled 76. Numerous examples of extra-intimal spaces are shown in figs. 105 to 147. Perhaps the best figure of an extra-intimal space is No. 111A. Figures 148 to 158 are lymphatics.

The theory of the origin of lymphatics from extra-intimal spaces has been especially described in an article by Huntington in 1910 (55). In a diagram on page 409 he gives his idea that a space which forms around a degenerating vein eventually includes the entire vein, which then disappears so that the lymphatic is left with a wall of mesenchyme.

There are numerous points which rule out the theory of the origin of the lymphatics by extra-intimal spaces. (1) These spaces can be varied at will by changing the fixation. In my experience they are much more common with lymphatics than with veins. We have sections of adult human tonsils, in which all surrounding lymphatics have extra-intimal spaces. In the human embryo, 460, the large jugular segment of the thoracic duct has one area where the endothelium has sagged from the surrounding tissue. There is, I think, no question but that the extra-intimal spaces are artefacts. This is made the more certain by the fact that they do not occur in the living tadpole's tail, but are found along the veins after fixation.

(2) In connection with the presentation of the extra-intimal spaces, Huntington gives no proof whatever that the structures he figures are degenerating veins. Most of the extra-intimal spaces he shows are along the line of the pulmonary and cardiac lymphatics arising from the jugular sacs. I think it probable from their size and position that the structures he is dealing with are shrunken lymphatics and not veins. The pulmonary and cardiac lymphatics (fig. 13) will, when reconstructed, give the same appearance of isolated lymphatic vessels as all other lymphatic trunks.

(3) The third point against the theory that lymphatics grow by extra-intimal spaces is this: the growing lymphatic tip always keeps as far as possible from the blood capillaries, just as in the adult the ultimate lymphatic capillaries are as far as possible from the blood capillaries; as, for example, in the relation of the central lacteals and peripheral blood capillaries in the villus. The point of the avoidance of the blood capillaries and of the mesenchyme cells as well by the growing lymphatic tip has been well described by Clark (26). Moreover, in certain tadpoles the lymphatics grow out ahead of the blood capillaries where no blood capillaries have ever been. Therefore, lymphatics do not grow by extra-intimal spaces, for extra-intimal spaces are artefacts; there is no evidence that lymphatics follow degenerating veins and on the other hand the growing lymphatic tip, far from following the blood capillaries, avoids them as much as possible.

3. GROWTH OF LYMPHATICS BY THE ADDITION OF TISSUE SPACES.

With these various structures as anlagen of lymphatic capillaries, Huntington and McClure have included certain of the tissue spaces lying along the course of the lymphatics. This theory Huntington

brought out especially in a study of the lymphatic system in reptiles (57). After a confused account of the origin of the anterior lymph hearts he describes the development of the peripheral lymph vessels out of tissue spaces. He says that whereas in mammals the greater number of the lymphatic vessels come from extra-intimal spaces (57, p. 272) in reptiles, on the other hand, the lymphatics come from tissue spaces without relation to the veins.

The theory that in the pathway of developing lymphatic vessels certain tissue spaces enlarge and are added onto the growing tips has been worked out by two pupils of McClure, Kampmeier (66a) and Stromsen (146a), both of whom published their work in 1912.

Kampmeier's first article (66) is a preliminary report of the second (66a). He has worked on the development of the thoracic duct in the pig and bases most of his conclusions on a reconstruction of a specimen of mine. Kampmeier thinks that the thoracic duct develops in an anterior-posterior direction by the addition of certain connective tissue spaces which enlarge in the pathway of a developing vessel. This is a return to the view of the earlier embryologists, except that they believed that the growth was from the periphery toward the center.

To a certain extent Kampmeier still holds to the theory of lymphatics from extra-intimal spaces; that is, he believes that a part of the thoracic duct follows veins that are degenerating. A vein which lies in the pathway of a developing lymphatic vessel he calls a "veno-lymphatic." This is a different use of the term from that of Huntington and McClure, who used the term to mean a vein which was transformed into a lymphatic vessel. This latter use of the term can well be given up, since veins do not become lymphatics. The replacing of degenerating veins Kampmeier does not regard as a fundamental process in the development of lymphatics, since they may or may not follow such veins. He thinks that in the development of the thoracic duct about half the duct comes from extra-intimal spaces (66a, p. 434). It is interesting to note in connection with the degeneration of veins in Kampmeier's work that he speaks of the plexus of veins medial to the nervous sympatheticus, fig. 11 (or fig. 11 in 66a), as veno-lymphatics; that is, as veins which disappear as the thoracic duct forms, and yet this same plexus of veins can be readily injected in embryo pigs 27 to 30 mm. long; stages in which the thoracic duct is well formed. It is true that the blood vessels may not show in uninjected specimens, just as the plexus of blood capillaries around

the air cells of an adult lung cannot be reconstructed from sections of uninjected specimens. The degeneration of blood vessels can only be followed by means of complete injections of different stages.

Kampmeier finds another example of lymph vessels which grow by the extra-intimal replacement of veins in the region where the thoracic duct empties into the jugular sac. In his fig. 8 from an embryo 20 mm. long he shows vessels in which there is a considerable sagging of the endothelium from the surrounding tissue, making the so-called extra-intimal spaces. Kampmeier's embryo measuring 20 mm. is about the same stage as mine, measuring 23 mm., since mine was measured before fixation, and hence his figure can be compared with fig. 12, in which a lymphatic injection makes it easy to separate veins from lymphatics. As Hoyer says (49b, p. 536): "Den Verlauf der Lymphgefäße nur an nicht injizierten Serienschnitten zu verfolgen, ist eine mühevoll und zeitraubende Arbeit, deren Ergebnisse, wie wir uns selbst überzeugt haben, höchstens nur für grössere Lymphstämme, deren Lage man kennt, als sicher zu bezeichnen sind. Ist der Verlauf von Gefässen und deren Verästelung noch unbekannt, so lassen sich auf Grund von Serienschnitten nur Vermutungen über Verlauf und Verästelung derselben aufstellen. Erst wenn man über Gefässe und ihre Aeste mit Injektionsmasse gefüllt vor sich sieht, erhält man einen guten Einblick in die Verteilung derselben, deren Verfolgung selbst an nicht injizierten Serienschnitten dann keine wesentlichen Schwierigkeiten mehr bietet." Although it is not possible to be entirely sure in regard to Kampmeier's sections, yet it is at least probable that he is dealing in large part with lymphatics and not with veins at all. The sagging of the endothelium from the surrounding tissue I have found more often with lymphatics than with veins, and yet in tissues fixed in Zeuker's fluid, which Kampmeier uses exclusively, it may occur in any of the veins and even in the aorta. Kampmeier says, with reference to this group of vessels (fig. 8 and fig. 29 in 66a, p. 460), that they are mesenchymal, perivascular spaces into which the jugular sac opens freely; that is, he thinks that the jugular sac in an embryo 20 mm. long opens freely into the tissue spaces. This point is easy to disprove, for in contrast to the early stages of the thoracic duct the jugular sac in this stage is easy to inject. If this were true then every injection of the sac in this stage would show extravasations. This is, however, not so. In fig. 7 is an injection of the sac in an embryo 18 mm. long and I can

... many injections that the vessel is dead and is a closed vessel. ... demonstrated that this upper ... generating veins.

... work is not that some ... veins, but that they develop ... of this theory he uses a ... mine. Notwithstanding the ... which has yet been made in a ... does not hesitate to call it ... through the jugular lymph sac, ... the thoracic duct. At a cer- ... an extravasation (Kampmeier, ... position in the next section ... It is therefore merely an ... vessel was actually connected ... say, there is as much evidence ... who has had experience with ... at the first injection in a new ... which Kampmeier has shown ... by endothelium; that is, ... (p. 76) has made us familiar; ... which Kampmeier and Strom-

8

... vertebral lymphatics in turtles ... zone there are enlarged ... to become lymphatics. Kamp- ... tissue spaces which are going to ... all incipient lymphatic an- ... in position or spaces fol- ... the discarded pathways of ... different from either an ... (Kampmeier 66a, p. 430). Strom- ... select tissue spaces which are ... point, that such enlarged tis- ... developing lymphatics. These ... anyone who has access to serial ... were not the enlarged tissue ... as lymphatics? Good examples

of such spaces are the constantly occurring spaces in the parietal pleura on either side of the vertebra. They are always present in the stages in which the thoracic duct is developing; they are adjacent to the vascular zone just internal to the ribs and they never have any relation to lymphatics. A definite, constant group of such spaces is also to be found in the subcutaneous tissue of the mid-dorsal line of certain stages and they never become lymphatics. The pia-arachnoid and perineural spaces likewise never become lymphatics. In fact, large as well as small groups of such spaces are a constant occurrence in sections of embryos; sometimes near lymphatics and sometimes far

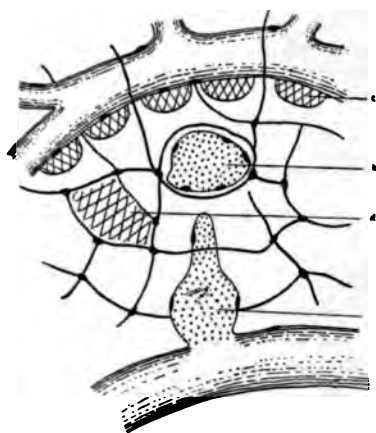


FIG. 19.—Diagram to show some of the various structures which Huntington and McClure have described as lymphatics. a. = true lymphatic capillaries; b. = Mayer-Lewis anlagen, also true lymphatic capillaries; c. = extra-intimal hypothetical lymphatic capillaries; d. = tissue spaces. The perineural spaces are not shown. The veins are striped, lymphatic capillaries dotted, and hypothetical lymphatic capillaries are cross-hatched.

from them. The selection of certain tissue spaces as destined to become lymphatics is an arbitrary selection and brings us back to the confused standpoint of the earlier embryologists; a standpoint which would indeed justify the view of Bartels that the question of the relation of the lymphatics to the tissue spaces is “eine philosophische, keine anatomische Frage.”

The difficulty of finding out the method of growth by observing sections is best illustrated by following the different theories of their growth in the pages of Huntington and McClure. Some of these methods are shown in the diagram, fig. 19. This development of the

subject has been necessarily confusing to those who have not followed the subject carefully. However, now that the origin and method of growth of the lymphatic system has been cleared up, so that the fundamental morphology is understood, the controversy has had this great value, that it has brought up for analysis and discussion every conceivable method of growth. Lymphatics do not arise as dilated peripheral tissue spaces after the manner of the coelom as the earlier embryologists thought; they do not grow by the addition of hollow connective tissue cells, as Schwann and Virchow thought; they do not rise as perineural spaces, nor by fenestration of a vein, nor by extra-intimal clefts, nor by the progressive addition of connective tissue spaces, nor by the addition of detached blood vessels, but they bud from the veins and grow by the sprouting of their endothelial wall.

IX. CONCLUSIONS.

The most important result of this study on the morphology of the lymphatic system is the emphasis it throws on the importance of endothelium as a tissue. The angioblast is one of the early tissues to be differentiated; it is not an inert lining for vessels, but an actively growing functioning tissue. In its place of growth it is a syncytium of actively amœboid protoplasm. Mollier (99) has shown that in the spleen it may be reticular; Mall (86 and 87) has shown that it may give rise to reticulum. Undoubtedly the further development of our knowledge of endothelium depends on the development of the new experimental anatomy.

The lymphatic endothelium buds off from the veins. It is always a little different in appearance from the endothelium of the veins, and the lymphatic capillary is different in size and form from the blood capillaries. The growing lymphatic tip has the remarkable characteristic that it avoids the blood capillaries, while it is attracted by other lymphatic capillaries.

Endothelium is the essential tissue of the lymphatic system. In the lower vertebrates lymph hearts are formed by the addition of striated muscle to primary lymph sacs. In the higher forms lymph glands are formed by the development of lymphocytes around the ducts. This takes place not only in the wall of the primary lymph sacs, but along plexuses of ducts, so that there are primary and secondary lymph glands.

The fundamental morphology of the lymphatic system has been established, but there remain many gaps in our knowledge of the system as a whole. The deep lymphatics in amphibia and reptilia are almost unknown; in fact a complete account of the lymphatic systems in both of these groups would be of great value. In birds the origin of the iliac lymphatics and the growth of the thoracic duct would be most interesting. In mammals the gaps in our knowledge are especially in regard to the development of the lymphatic system within many of the organs; for example, the heart and lungs, the liver, spleen, kidneys and reproductive organs. A study of the embryology of the lymphatic system makes it more certain that the central nervous system has no lymphatics. The lymphatics invade the body, but not completely; the nervous system is a permanent non-lymphatic area.

It is now possible to define the lymphatics. Lymphatic capillaries are tubes of endothelium; they are derived from the endothelium of the veins, and they have the same relation to tissue spaces as have blood capillaries.

LITERATURE.

1. ABEL, J. J.: On the action of drugs and the function of the anterior lymph hearts in cardiectomized frogs. *Journ. of Phar. and Exp. Ther.*, Vol. 3, 1912.
2. ALLEN, W. F.: Distribution of the lymphatics in the head, and in the dorsal, pectoral, and ventral fins of *Scorpaenichthys marmoratus*. *Proc. Wash. Acad. of Science*, Vol. 7, 1906.
3. ——— Distribution of the subcutaneous vessels in the tail region of *Lepisosteus*. *Amer. Journ. of Anat.*, Vol. 8, 1908; *Anat. Record*, Vol. 2, 1908.
4. ——— Distribution of the lymphatics in the tail region of *Scorpaenichthys marmoratus*. *Amer. Journ. of Anat.*, Vol. 11, 1910-1911.
5. ARNOLD, J.: Experimentelle Untersuchungen ueber die Entwicklung der Blutcapillaren. *Arch. für path. Anat.*, Bd. 53, 1871 and Bd. 54, 1872.
6. ——— Ueber die Beziehung der Blut- und Lymphgefäße zu den Saftkanälen. *Arch. für path. Anat.*, Bd. 63, 1875.
7. v. BAER: *Entwicklungsgeschichte der Tiere*. I. Teil, 1828. II. Teil, 1837.

8. BAETJER, WALTER A.: On the origin of the mesenteric sac and thoracic duct in the embryo pig. *Amer. Journ. of Anat.*, Vol. 8, 1908.
9. BARAŃSKI, J.: Die Entwicklung der hinteren Lymphherzen der Unke (Bombinator). *Bull. de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématiques et Naturelles. Série B. Sciences Naturelles*, 1911.
10. BARTELS, P.: *Das Lymphgefäßsystem. Handbuch der Anatomie des Menschen. Von Bardeleben*, 1909.
11. BAUM, H.: Können Lymphgefäße direkt in Venen einmünden? *Anat. Anz.*, Bd. 39, 1911.
12. BICHAT: *Anatomie Générale*, 1818.
13. BOERHAVE: Quoted from Bichat., *Anat. Gén.*, S. 596.
14. BONNET, R.: *Grundriss der Entwicklungsgeschichte*. Berlin, 1891; Zweite Auflage, 1912.
15. BREMER, H. L.: The development of the aorta and aortic arches in rabbits. *Amer. Journ. of Anat.*, Vol. 13, 1912.
16. BRESCHET, G.: *Le Système Lymphatique*. Paris, 1836.
17. BUDGE, ALBRECHT: Neue Mittheilungen ueber die Lymphgefäße der Leber. *Königl. Sachs. Gesellsch. d. Wiss. zu Leipzig. Math.-Phys. Classe. Berichte 27-30, 1875-80*, S. 161.
18. ——— Die Lymphwurzeln der Knochen. *Arch. f. mik. Anat.* 13, 1877.
19. ——— Ueber ein Kanalsystem in Mesoderm von Hühnerembryonen. *Arch. für Anat. und Phys., Anat. Abth.*, 1880.
20. ——— Ueber das dem zweiten Blutkreislaufs entsprechende Lymphgefäßsystem bei Hühnerembryonen. *Centralbl. f. d. med. Wissensch.*, 1881.
21. ——— Ueber Lymphherzen bei Hühnerembryonen. *Arch. für Anat. und Phys., Anat. Abth.*, 1882.
22. ——— Untersuchungen über die Entwicklung des Lymphsystems beim Hühnerembryo. *Arch. für Anat. u. Phys., Anat. Abth.*, 1887.
23. CRUIKSHANK, W.: *The anatomy of the absorbing vessels of the human body*. London, 1790.
24. CLARK, A. H.: On the fate of the jugular lymph sacs, and the development of the lymph channels in the neck of the pig. *Amer. Journ. of Anat.*, Vol. 14, 1912.
25. CLARK, E. R.: Observations on living growing lymphatics in the tail of the frog larva. *Anat. Record*, Vol. 3, 1909.

26. ——— An examination of the methods used in the study of the development of the lymphatic system. *Anat. Record*, Vol. 5, 1911.
27. CLARK, ELEANOR LINTON: Injection and reconstruction of the jugular lymph sac in the chick. *Anat. Record*, Vol. 6, 1912.
28. ——— General observations on early superficial lymphatics in chick embryos. *Anat. Record*, Vol. 6, 1912.
29. CLARK, ELIOT R., and ELEANOR L.: Observations on the development of the earliest lymphatics in the region of the posterior lymph heart in living chick embryos. *Anat. Record*, Vol. 6, 1912.
30. CLARK, ELIOT R.: Further observations on living growing lymphatics: their relation to the mesenchyme cells. *Amer. Journ. of Anat.*, Vol. 13, 1912.
31. DRASCH, O.: Die Bildung der Somatopleura und der Gefäße beim Hühnchen. *Anat. Anz.* 9, 1894; S. 567.
32. DZIURZYNSKI, A.: Untersuchungen ueber die Regeneration der Blut und Lymphgefäße im Schwanze von Froschlarven. *Bull. de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématiques et Naturelles. Série B. Sciences Naturelles*, 1911.
33. EVANS, H. M.: On the development of the aorta, cardinal and umbilical veins, and the other blood vessels of vertebrate embryos from capillaries. *Anat. Record*, Vol. 3, 1909.
34. ——— On the earliest blood vessels in the anterior limb buds of birds and their relation to the primary subclavian artery. *Amer. Journ. of Anat.*, Vol. 9, 1909.
35. ——— Die Entwicklung des Blutgefäßsystems. *Handbuch der Entwicklungsgeschichte des Menschen. Keibel und Mall. Bd. 2. Leipzig*, 1911.
36. FAVARO, G.: Ricerche anatomo-embriologiche intorno alla circolazione caudale ed ai cuori linfatici posteriori degli Anfibi con particolare riguardo agli Urodeli. *Atti del l'Acad. Sc. veneto-trentino-istriana. Cl. 1*, 1906.
- 36a. ——— Ricerche intorno all morfologia ed all sviluppo dei vasi, seni e cuori caudali nei Ciclostomi e nei Pesci. *Atti del Reale Istituto Veneto di sc. lett. ed arti. Venezia. T. 65*, 1906.

37. GOETTE: Entwicklungsgeschichte der Unke als Grundlage einer vergleichenden Morphologie der Wirbelthiere. Leipzig, 1874.
38. GOLDFINGER, GIZELLA: Ueber die Entwicklung der Lymphsäcke in den hinteren Extremitäten des Frosches. Bull. de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématiques et Naturelles, 1907.
39. GULLUND: The development of lymphatic glands. Journ. of Path. and Bact., Vol. 11, 1894.
40. HAHN, H.: Experimentelle Studien über die Entstehung des Blutes und der ersten Gefäße beim Hühnchen. Archiv für Entwicklungsmechanik der Organismen. Bd. 27, 1909.
41. HALLER: Elementa physiologiæ corporis humani. T. 1. 1757-63, s. 110.
42. HELLY, KONRAD: Hæmolymphdrüsen. Ergebnisse der Anatomie und Entwicklungsgeschichte. Bd. 12, 1902.
43. HEUER, GEORGE J.: The development of the lymphatics in the small intestine of the pig. Amer. Journ. of Anat., Vol. 9, 1909.
44. HIS, W.: Ueber die Wurzeln der Lymphgefäße in den Häuten des Körpers und über die Theorien der Lymphbildung. Zeitschr. f. wissensch. Zoölogie, XII, 1863.
45. ——— Ueber das Epithel der Lymphgefäßwurzeln und ueber die von Recklinghausenschen Saftcanälchen. Zeitsch. f. wissensch. Zoologie, XIII, 1863.
46. ——— Untersuchungen ueber die erste Anlage des Wirbeltierleibes. Leipzig, 1868.
47. ——— Lecithoblast und Angioblast der Wirbeltiere. Abhandl. der mathematisch-physischen Klasse der Königl. Sächs. Gesellsch. d. Wissenschaften. Bd. 26, 1900.
48. HOYER, H.: Ein Beitrag zur Histologie bindegewebiger Gebilde. Arch. für Anat. und Phys. und wiss. Medicin, 1865.
49. ——— Untersuchungen ueber das Lymphgefäßsystem der Froschlarven. Bull. de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématiques et Naturelles, 1908.
- 49a. ——— Zur Entwicklung der Lymphgefäße bei Wirbeltieren. Verhandl. d. VIII Internat. Zool. Kongr. zu Graz. (vom. 15-20, 8, 1910), 1912.

- 49b. ——— Und Udziela, S., Untersuchungen ueber das Lymphgefäßsystem von Salamanderlarven. *Morph. Jahrb.*, Bd. 44, 1912.
50. HUNTER, W.: *Medical Commentaries*. Deutsch. von. Kühne als medicinisch-chirurgische Beobachtungen. Leipzig, 1785. Bd. 2.
51. HUNTINGTON, G. S., and McCURE, C. F. W.: The development of the main lymph channels of the cat in their relations to the venous system. *Anat. Record*, Vol. 1, 1906-1908.
52. ——— The anatomy and development of the jugular lymph sacs in the domestic cat. *Anat. Record*, Vol. 2, 1908.
53. HUNTINGTON, G. S.: The genetic interpretation of the development of the mammalian lymphatic system. *Anat. Record*, Vol. 2, 1908.
54. HUNTINGTON, G. S., and McCURE, C. F. W.: The anatomy and development of the jugular lymph sacs in the domestic cat. *Amer. Journ. of Anat.*, Vol. 10, 1910.
55. HUNTINGTON, G. S.: The phylogenetic relations of the lymphatic and blood vascular system in vertebrates, p. 1, and The genetic principles of the development of the systemic lymphatic vessels in the mammalian embryo, p. 339. *Anat. Record*, Vol. 4, 1910.
56. ——— Ueber die Histogenese des lymphatischen Systems beim Säugerembryo. *Verhandl. der Anat. Gesellsch.*, 1910.
57. ——— The development of the lymphatic system in reptiles. *Anat. Record*, Vol. 5, 1911.
58. ——— The anatomy and development of the systemic lymphatic vessels in the domestic cat. *Memoires of the Wistar Institute of Anatomy and Biology*, No. 1, 1911.
59. JOLLY, J.: Recherches sur les ganglions lymphatiques des oiseaux. *Arch. d'Anat. microsc.*, T. 11, 1910.
60. JOURDAIN, M. S.: Sur l'existence d'une circulation lymphatique chez les Pleurometes. *Comptes Rendus de l'Académie des Sciences*, 90, 1880, p. 1430.
61. JOURDAIN, M. L.: Sur le système lymphatique des têtards et grenouilles. *Comptes Rendus de l'Académie des Sciences*, 96, 1883.
62. JOSEFROW, G.: Zur Lehre von dem Lymphgefäßsystem der Froschlarve, des Frosches und der Eidechse. *Ref. in Schwalbe's Jahresber.*, 1905, III, S. 353.

63. JOSSIFROW, S. M.: Ueber die Bedeutung des Cisterna chyli für die Lymphbewegung. Russki Wratsch. Jahrg. II. N. 29, S. 1909-1910.
64. JOSSIVOW, G.: Sur le système lymphatique du têtard, de la grenouille et du lézard. 1 Taf. Mém. l'Acad. Imp. des Sc. St. Petersbourg. Sér. 8. T. 15. N. 11, p. 20.
65. ——— Der Anfang des Ductus thoracicus und seine Erweiterung. Trav. Soc. Méd. scientif. et. hyg. Charkoff. S. 1-9.
66. KAMPMEIER, O. F.: The value of the injection method in the study of lymphatic development. Anat. Record, Vol. 6, 1912.
- 66a. ——— The development of the thoracic duct in the pig. Amer. Journ. of Anat., Vol. 13, 1912.
67. KOELLIKER: Sur le developpement des tissues chez les batraciens. Annales des Sciences Naturelles, IIIe Serie, 1846.
68. ——— Ueber die Blutkörperchen eines menschlichen Embryo und die Entwicklung der Blutkörperchen bei Säugetieren. Zeitschrift f. rationelle Medizin. Henle und Pfeufer. Bd. IV, 1846.
69. ——— Entwicklungsgeschichte des Menschen und der höheren Tiere. Zweite Auflage. Leipzig 1876, S. 158.
70. ——— Grundriss der Entwicklungsgeschichte des Menschen und der höheren Tiere. 2 Aufl. Leipzig 1884.
71. ——— Histologische Studien an Batrachierlarven. Zeitschr. f. wissensch, Zoologie. 43, 1886.
72. KLING: Studien über die Entwicklung der Lymphdrüsen beim Menschen. Upsala Läkareförenings Föranslinger. 1903, and Arch. f. mikr. Anat. u. Entwicklungsgesch. Bd. 63, 1902.
73. KNOWER, H. McE.: Effects of early removal of the heart and arrest of circulation on the development of frog embryos. Anat. Record, Vol. 1, 1907.
74. ——— The origin and development of the anterior lymph hearts and the subcutaneous lymph sacs in the frog. Anat. Record, Vol. 2, 1908.
75. LANGER, C.: Ueber das Lymphgefäßsystem des Frosches. Die Lymphgefäße im Schwanz der Batrachierlarven. Sitzungsber. d. k. Akad. d. Wissensch. Bd. 58, 1868.
76. LEWIS, F. T.: The development of the lymphatic system in rabbits. Amer. Journ. of Anat., Vol. 5, 1906.

77. ——— The first lymph glands in rabbit and human embryos. *Anat. Record*, Vol. 3, 1909.
78. ——— On the cervical veins and lymphatics in four human embryos, with an interpretation of anomalies of the subclavian and jugular veins in the adult. *Amer. Journ. of Anat.*, Vol. 9, 1909.
79. MACCALLUM, W. G.: Die Beziehung der Lymphgefäße zum Bindegewebe. *Arch. für Anat. und Phys.*, Anat. Abth., 1902.
80. MALL, F. P.: Development of the ventral abdominal walls in man. *Journ. of Morphology*, Vol. 14, No. 2, 1898.
81. ——— The cœlom. *Reference Handbook of the Medical Sciences*. William Wood & Co., New York. Second Edition. 1900-1904.
82. ——— Development of the internal mammary and deep epigastric arteries in man. *John Hopkins Hospital Bulletin*, 1898.
83. ——— On the origin of the lymphatics in the liver. *Proceedings of the Association of American Anatomists*, 1900.
84. ——— Development of the connective tissues from the connective syncytium. *Amer. Journ. of Anat.*, Vol. 1, 1901-1902, p. 354.
85. ——— On the development of the blood vessels of the brain in the human embryo. *Amer. Journ. of Anat.*, Vol. 4, 1905.
86. ——— A study of the structural unit of the liver. *Amer. Journ. of Anat.*, Vol. 5, 1906.
87. ——— On the development of the human heart. *Amer. Journ. of Anat.*, Vol. 13, 1912, p. 258.
- 87a. MARCUS, H.: Beiträge zur Kenntniss der Gymnophionen. *Morphologisches Jahrb.* Bd. 38, 1908.
88. MAYER, J.: Ueber die Neubildung von Blutgefässen in plastischen Exsudaten seröser Membranen und in Hautwunden. *Annal. d. Charité*, IV, p. 41-140.
89. MAYER, SIGMUND: Ueber die blutleeren Gefässe im Schwanz der Batrachierlarven. *Sitzungsberichte, der math. naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien*. Bd. 91, III Abth., 1885, S. 204.
- 89a. MEYER, F.: *Systema amphibiorum lymphaticum disquisitionibus novis examinatio*. Diss. inaug., Berolini, 1845. Quoted from Hoyer (49b).

90. MAXIMOW, A.: Untersuchungen ueber Blut und Bindegewebe. Archiv für mikroskopische Anatomie und Entwicklungsgeschichte, Bd. 73, 1909.
91. McCLURE, C. F. W.: The development of the thoracic and right lymphatic ducts in the domestic cat (*Felis domestica*). Anat. Anz., Bd. 32, 1908.
92. McCLURE, C. F. W., and SILVESTER, C. F.: A comparative study of the lymphatico-venous systems in adult mammals. Anat. Record, Vol. 3, 1909.
93. McCLURE, C. F. W.: The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat (*Felis domestica*). Verhandl. der Anat. Gesellsch., 1910.
94. ——— A few remarks relative to Mr. Kampmeier's paper on the value of the injection method in the study of lymphatic development. Anat. Record, Vol. 6, 1912.
95. MELTZER, S. J.: The distribution of solutions in cardiectomized frogs. Journ. of Exper. Med., Vol. 13, 1911.
96. MIERZEJEWSKI, LADISLAS: Beitrag zur Entwicklung des Lymphgefäßsystems der Vögel. Bull. de l'Acad. des Sciences de Cracovie. Classe des sciences mathematiques et naturelles, 1909.
97. MILLER, A. M.: The development of the jugular lymph sac in birds. Amer. Journ. of Anat., Vol. 12, 1912.
- 97a. ——— Histogenesis and morphogenesis of the thoracic duct in the chick; development of blood cells and their passage to the blood stream via the thoracic duct. Amer. Journ. of Anat., Vol. 15, 1913.
98. MINOT: Die Entwicklung des Blutes. Handbuch der Entwicklungsgeschichte des Menschen. Keibel und Mall. Bd. II, Leipzig 1911.
99. MOLLIER, S.: Ueber den Bau der kapillaren Milzvenen (Milzsinus). Arch. f. mikr. Anat., Bd. 76, 1911.
100. NUCK, A.: Adenographia curiosa. Leidæ, 1691.
101. PANDER: Beiträge zur Entwicklungsgeschichte des Hühnchens im Ei. Würzburg, 1817.
102. PANNIZZA, B.: Osservazionni antropo-zootomico fysiologiche. Pavia, 1830.
103. ——— Sopra il sistema linfatico dei rettili, ricerche zootomiche. Pavia, 1833.

104. PENSA, A.: Della struttura e dello sviluppo dei gangli linfatici degli uccelli (*Ancer domesticus*). Ric. f. n. Lab. di Anat. norm. d. R. Univ. di Roma. Vol. 12, 1906-07. Tav. 15. Fig. 2.
105. ——— Studio sulla morfologia e sulla topografia della cisterna chili e del ductus thoracicus nell'uomo ed in altri mammiferi. Ric. f. n. Lab. di Anat. norm. d. R. Univ. di Roma. Vol. 14, 1908-09. Tav. 2.
106. PLATNER, E. A.: Einige Beobachtungen ueber die Bildung der Capillargefäße. Müller's Archiv f. Anat. Phys. und wissensch. Medicin, 1844, S. 525-526.
107. POLINSKI, W.: Untersuchungen ueber die Entwicklung der subcutanen Lymphgefäße der Säuger, in sonderheit des Rindes. Bull. de l'Acad. des Sciences de Cracovie. Classe des Sciences Math. et Naturelles, Serie B, 1910.
108. PRÉVOST ET LEBERT: Mémoire sur la formation des organes de la circulation et du sang dans les batraciens. Ann. des sciences nat. 3 Serie, 1844. T. I, II et III.
109. RADWANSKA, M.: Die vorderen Lymphherzen der Frösche. Bull. intern. Ac. Sc. Cracovie, 1906.
110. RANVIER, L.: Morphologie du système lymphatique. De l'origine des lymphatiques dans la peau de la grenouille. C. R. Ac. des sc. T. 120, 1895, p. 132.
111. ——— Structure des ganglions lymphatiques du porc. C. R. Ac. des sc. T. 121, 1895, p. 800.
112. ——— Étude morphologique des capillaires lymphatiques des mammifères. C. R. Ac. des sc. T. 121, 1895, p. 856.
113. ——— Développement des vaisseaux lymphatiques. C. R. Ac. des sc. T. 121, 1895, p. 1105.
114. ——— Aberration et régression des lymphatiques en voie de développement. C. R. Ac. des sc. T. 122, 1896, p. 578.
115. ——— Des lymphatiques de la villosité intestinale chez la rat et le lapin. C. R. Ac. des sc. T. 123, 1896, p. 923.
116. ——— La théorie de la confluence des lymphatiques et la forphologie du système lymphatique de la grenouille. C. R. Ac. des sc. T. 123, 1896, p. 970.
117. ——— La théorie de la confluence des lymphatiques et la développement des ganglions lymphatiques. C. R. Ac. des sc. T. 123, 1896, p. 1038.

118. ——— Morphologie et développement des vaisseaux lymphatiques chez les mammifères. *Archives d'Anatomie microscopique*. T. 1, 1897, p. 69 et p. 137.
119. VON RECKLINGHAUSEN: Die Lymphgefäße und ihre Beziehung zum Bindegewebe. Berlin, 1862.
120. ——— Das Lymphsystem. Stricker's Handbuch der Lehre von den Geweben. Leipzig, 1871-72.
121. REICHERT, K. B.: Studien des physiologischen Instituts zu Breslau. Leipzig, 1858.
122. ——— Entwicklungsleben im Wirbelthierreich. Berlin, 1840 und 1857.
123. ——— Entwicklung des Meerschweinchens. Abh. der Berliner Akad., 1862.
124. REMAK, R.: Untersuchungen ueber die Entwicklung der Wirbeltiere. Berlin, 1850-55.
125. ——— Ueber blutleere Gefäße (Lymphgefäße) im Schwanze der Froschlarve. Müller's Archiv f. Anat. Phys. und wiss. Medicin. 1850. S. 102 und S. 182.
126. ROUGET, CHARLES: Mémoire sur les développement, la structure et les propriétés physiologiques des capillaires sanguins et lymphatiques. *Arch de Phys.*, V, 1873.
127. RÜCKERT: Ueber die Abstammung der blutigen Gefässanlagen beim Huhn, und ueber die Entstehung des Randsinus beim Huhn und bei Torpedo. *Sitzungsber. d. k. bayer. Akad. d. Wissensch. zu München*, 1902.
128. RÜCKERT, J.; und MOLLIER, S.: Die erste Entstehung der Gefäße und des Blutes bei Wirbeltieren. *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere*. Hertwig, Bd. I. T. 1, 1906.
129. SABIN, F. R.: On the origin of the lymphatic system from the veins and the development of the lymph hearts and thoracic duct in the pig. *Amer. Journ. of Anat.*, Vol. 1, 1901-02.
130. ——— On the development of the superficial lymphatics in the skin of the pig. *Amer. Journ. of Anat.*, Vol. 3, 1904.
131. ——— The development of the lymphatic nodes in the pig and their relation to the lymph hearts. *Amer. Journ. of Anat.*, Vol. 4, 1905.
132. ——— Further evidence on the origin of the lymphatic endothelium of the blood vascular system. *Anat. Record*, Vol. 2, 1908.

133. ——— The lymphatic system in human embryo, with a consideration of the morphology of the system as a whole. *Amer. Journ. of Anat.*, Vol. 9, 1909.
134. ——— Die Entwicklung des Lymphgefäßsystems. *Handbuch der Entwicklungsgeschichte des Menschen.* Keibel und Mall, Vol. 2, Leipzig, 1911. *Manual of Human Embryology,* Keibel und Mall. J. B. Lippincott Co., 1912.
135. ——— A critical study of the evidence presented in several recent articles on the development of the lymphatic system. *Anat. Record*, Vol. V, 1911.
136. ——— On the origin of the abdominal lymphatics in mammals from the vena cava and the renal veins. *Anat. Record*, Vol. 6, 1912.
- 136a. ——— Der Ursprung und die Entwicklung des Lymphgefäßsystems. *Ergebnisse der Anatomie und Entwicklungsgeschichte*, 1913.
137. SALA, L.: Sullo sviluppo dei cuori limfatici e dei dotti toracici nell'embrione di pollo. *Ricerche Lab. di Anat. Norm. d. r. Univ. di Roma.* Vol. 7, 1899-1900.
138. SAXER, FR.: Ueber die Entwicklung und den Bau der normalen Lymphdrüsen und die Entstehung der roten und weissen Blutkörperchen. *Anat. Hefte.* Bd. 6, 1896.
139. SCHWANN: *Structur und Wachstum der Thiere und Pflanzen.* Berlin, 1839.
140. SILVESTER, CHARLES F.: On the presence of permanent communications between the lymphatic and venous systems at the level of the renal veins in adult South American monkeys. *Amer. Journ. of Anat.*, Vol. 12, 1912.
141. SMITH, HELEN W.: On the development of the superficial veins in the body wall in the pig. *Amer. Journ. of Anat.*, Vol. 9, 1909.
142. STANNIUS: Ueber Lymphherzen der Vögel. *Müller's Archiv für Anat., Phys. und wiss. Medicin*, 1843.
143. STRICKER, S.: Studien ueber den Bau und das Leben der capillaren Blutgefässe. *Sitzungsbericht der Math.-Naturwiss. Klasse der k. Akad., Wien.* Bd. 52. Abth. 2, 1865, S. 379.
144. ——— Untersuchungen ueber die Contractilität der Kapillaren. *Med. Jahrb., Wien*, 1878.

145. STROMSEN, F. A.: A contribution to the anatomy and development of the posterior lymphatic heart of turtles. Publication 132 of the Carnegie Institution of Washington, 1910.
146. ——— On the relations between the mesenchymal spaces and the development of the posterior lymph hearts of turtles. *Anat. Record*, Vol. 5, 1911.
- 146a. ——— On the development of the prevertebral (thoracic) duct in turtles as indicated by a study of injected and uninjected embryos. *Anat. Record*, Vol. 6, 1912.
147. SZILY, A.: Ueber das Entstehen eines fibrillären Stützgewebes im Embryo und dessen Verhältniss zum Glaskörperfrage. *Anat. Hefte*. Bd. 35, 1908.
148. THOMA: Untersuchungen ueber die Histogenese und Histomechanik des Gefässsystems. Stuttgart, 1893.
149. TILNEY, F.: The development of the veins and lymphatics in *Tragulus meminna*, erleben. *Amer. Journ. of Anat.*, Vol. 13, 1912.
150. VIRCHOW: Die Cellularpathologie. Berlin, 1858.
151. WIELIKY, W.: Einige Beiträge zur Anatomie und Physiologie der Lymphherzen. Ref. in Hoffmann-Schwalbe's *Jahresber.*, 1884.
152. ——— Ueber die Anwesenheit vielzähliger Lymphherzen bei den Froschlarven. *Zool. Anz.* Bd. 9, 1886.
153. ——— Weitere Untersuchungen ueber die Lymphherzen und Lymphgefäße einiger Amphibien. Ref. in Hoffmann-Schwalbe's *Jahresb.*, 1889.
154. WOLFF: *Theoria Generationes* (Original 1759). Ausgabe von 1764.
155. WYSOTZKY, N. TH.: Materialien zur embryonalen Entwicklungsgeschichte der Gefäße. *Journ. f. norm. u. path. Histologie u. klin. Medicin.* Herausgegeben von M. Rudneff. St. Petersburg, 1875. Schwalbe und Hoffmann, *Jahresberichte*. 1876. S. 145.

SEPARATE MONOGRAPHS REPRINTED FROM THE JOHNS
HOPKINS HOSPITAL REPORTS.

- Studies in Dermatology. By T. C. GILCHRIST, M. D., and EMMER RISSMAN, M. D. 164 pages and 41 plates. Price, in paper, \$3.00.
- The Malarial Fevers of Baltimore. By W. S. THAYER, M. D., and J. HEWERTSON, M. D. And a Study of some Fatal Cases of Malaria. By LEWELLYS F. BARKER, M. B. 280 pages. Price, in paper, \$3.75.
- The Pathology of Toxalbumin Intoxications. By SIMON FRIENES, M. D. 150 pages with 4 lithographs. Price, in paper, \$2.00.
- Studies in Typhoid Fever, I, II, III. By WILLIAM OSLER, M. D., and others. Extracted from Vols. IV, V and VIII of the Reports. 757 pages. Price, in cloth, \$5.00.
- Pneumothorax. A Historical, Clinical, and Experimental Study. By CHARLES P. EMERSON, M. D. Price, in paper, \$4.00.

NEW SERIES.

- I. Free Thrombi and Bail-Thrombi in the Heart. By J. H. HEWITT, M. D. 32 pages and 20 illustrations. Price, in paper, \$1.00.
- II. Benzol as a Leucotoxin. By LAURENCE SELLING, M. D. 60 pages and 3 plates, 1 in colors. Price, in paper, \$1.00.
- III. Primary Carcinoma of the Liver. By M. C. WESTERNITZ, M. D. 42 pages. Price, in paper, 75 cents.
- IV. The Statistical Experience Data of The Johns Hopkins Hospital, Baltimore, Md., 1892-1911. By FREDERICK L. HOFFMAN, LL. D., F. S. S. 161 pages. Price, in paper, \$2.00.
- V. The Origin and Development of the Lymphatic System. By FLORENCE R. SANS. 94 pages. Price, in paper, \$2.00.