THE AMERICAN JOURNAL

OF

ANATOMY

VOLUME VI
1906-1907

THE AMERICAN JOURNAL OF ANATOMY
BALTIMORE, MD., U. S. A.

The Lord Baltimore Press
BALTIMORE, MD., U. S. A.

CONTENTS OF VOL. VI.

The first four numbers of The Anatomical Record *were issued with this volume of The American Journal of Anatomy.* The Proceedings of the Association of American Anatomists, *Twenty-first Session, December, 1906, and Twenty-second Session, March, 1907, are included in Nos. 3 and 4 of the* Record.

THE DEVELOPMENT OF THE LUNGS.

BY

JOSEPH MARSHALL FLINT, M. D.,

Professor of Anatomy in the University of California.

(*From the Hearst Anatomical Laboratory of the University of California.*)

With 4 Plates and 29 Text Figures.

It requires only a cursory inspection of the literature on the lungs to show the unsatisfactory state of our knowledge concerning the development of these organs. In the first place, the ontogeny and phylogeny of the mammalian lungs have stood in apparent conflict. There are, moreover, few features of their anatomy upon which there is any agreement among the various investigators who have contributed to this field. As a reworking of the entire subject has seemed desirable, the author was guided in choosing the pig, first of all, by the practically unlimited supply of the different embryonic stages and, secondly, by the fact that the artiodactyls possess in well developed form, all of the most discussed types of bronchi.

Methods.

For the study of the early stages of the development of the respiratory system, the Born reconstruction method was employed. Fruitful suggestions for its use have been obtained from the contributions of Bardeen and Huber, whose applications of the Born method have been followed in this study. Sections of a series of pigs were cut at 20 micra and stained in hæmatoxylin and congo red. The reconstructions were made at a magnification of 100 diameters. In order to obtain an accurate orientation of the subdivisions of the bronchi, the piling of the plates according to the external form of the lung was controlled by dissections of the lungs of a series of embryos of a corresponding age as those used for reconstruction after the method suggested by Minot. Liberal use has been made of the various corrosion methods to follow the evolution of the bronchial tree in pigs from 4 cm. to those of adult life. The use of Wood's metal and of celloidin corrosions gave fruitful results, although the majority of the stages were obtained by the use

of celluloid corrosions. For this purpose celluloid is dissolved in acetone and injected from aspiration bottles into the lungs through the trachea. Like the cellodin corrosions these were digested or macerated in concentrated hydrochloric acid. The advantage of celluloid over celloidin casts lies in the fact that the former, like Wood's metal, may be left in the air and handled freely without the disadvantages of the glycerine bath, which often makes it either difficult or impossible to study certain parts of the celloidin preparations. For the study of the development of the respiratory lobules a combination of celluloid and Wood's metal preparations proved most advantageous. Preparations of the entire embryonic lung cleared in oil of cloves were also found serviceable as control preparations for the reconstructions. They are, however, of doubtful value save for this purpose as the young dorsal and ventral buds on the stem bronchus are almost invisible until they have reached a considerable size.

The organogenesis was followed in a series of stained sections from embryos and lungs hardened in Zenker's fluid and stained by Mallory's method. At the period of birth the alveoli were distended by injecting them, under low pressure, with Zenker's fluid, thus obviating the obscure and uncertain pictures which are obtained when the lung is collapsed and contracted. In following the development of the epithelium, the well-known silver nitrate method has been used.

Review of the Literature.

To von Baer, **28**, we are indebted for the first description of the dedevelopment of the pulmonary apparatus. In the chick it consists of two small hollow swellings about the middle of the head gut, which appear on the third day. These projections give rise to the lungs, while the hollow cavities represent the rudiments of the bronchi although the trachea up to this time is unformed. On the fourth day the lungs, still in connection with the œsophagus, lie more ventralwards, but the bronchi in growing backwards have dilated into small sacs. Anteriorly, however, the bronchi join each other at an acute angle and terminate in a short canal, the anlage of the trachea which communicates with the œsophagus behind the pharynx. These observations were amplified by the work of Remak, **55**, Selenka, **66**, Götte, **67**, and especially His, **68**, who believes the larynx and trachea arise from a ventral groove in the head gut. Caudalwards, this structure has two lateral projections representing the rudiments of the bronchi which are bilateral and paired in contradistinction to the unpaired anlage of the larynx and trachea. Less in

accordance with our modern ideas on the development of the lungs are the papers of Rathke, 28, and Seessel, 77, while more recent contributions are those of Fischelis, 85, and Kastschenko, 87. The work of the latter has been especially emphasized by Weber and Buvignier, 03, who support his views on the serial homology of the lungs with the branchial pouches. They believe, from their work on the duck, that in birds as well as mammals the anlage of the lungs are paired derivatives of the respiratory tube. The lungs, therefore, while not representing actually existing branchial pouches, indicate the reappearance of endodermic evaginations of the head gut which has carried gills among the ancestors of vertebrates.

The study of the development of the amphibian and reptilian lung was taken up somewhat later when Rathke, 39, in Coluber natrix described its appearance from paired projections from the head gut. He states that the right lung increases in size until it is larger than the stomach while the left remains, in consequence of regressive changes, as a slight appendix of the trachea. Baumann, 02, in Tropidonotus natrix confirms these observations of Rathke by finding the right lung is three times larger than the left in an embryo 3 mm. long, while at 5 mm. it is some forty times larger. But he is inclined to believe, however, that the discrepancy in size is due to arrested development of the left lung sac rather than a true regressive process. Betrachians were studied by Remak, 55, who found the first rudiments as paired buds from the head gut passing laterally and caudally, while Götte, 75, describes the origin of the lungs in Anura from endodermal projections immediately behind the last branchial pouch. Götte, in Anura, suggested the possibility of transformed branchial pouches taking part in the formation of the lungs, before Kastschenko described the origin of the avian lung from the respiratory tube. Naturally, the observations of Götte, like those of Kastschenko, are supported by Weber and Buvignier, 03, while Götte, 04, himself, more recently reaffirms that theory. Greil, 05, however, who also worked on Anurans comes to the opposite conclusion from these investigators. Primitively the lungs appear, according to Greil, in the form of two bilaterally symmetrical grooves in the ventral wall of the heat gut about the time the first four gill pouches are formed. The fifth and sixth pouches appear later and are separated from the lung anlage by an appreciable space which is greater than the interval between the individual pouches. He concludes, therefore, that the gill pouches have nothing whatever to do with the formation of the lungs. In subsequent stages the pulmonary grooves deepen and are covered with a thickened splanchnopleure to form the primitive lung sac.

Between these structures a transverse gutter appears, while the portion of the head gut anterior to this, produced by the narrowing of its lateral walls, forms a longitudinally placed laryngo-tracheal groove, which gives rise to the trachea and larynx. The separation from the œsophagus then begins at the caudal extremity and proceeds forwards.

Among the earlier investigators there was an apparent unanimity of opinion that the subsequent differentiation of the amphibian and reptilian lung was due to a centripetal ingrowth of septa from the lung wall dividing and subdividing the primitive lung cavity into a series of smaller peripheral spaces. Furthermore, as early as the middle of the last century Leydig, 57, taught that the complicated lungs of the higher vertebrates represented a complex of a series of simpler lungs, or, in other words, that the infundibulum of the mammalian lung might be compared with an entire frog's lung with its parietal alveoli. Miller, 93, in a comparative study of the reptilian, avian, and mammalian lung, states that the complexity of the reptilian lung is due to a system of septum formation while the process of budding plays a secondary rôle. In the avian lung, however, budding becomes more important and septum formation is secondary. Thus Miller looked upon the avian lung as a transition stage between the reptilian lung with its septum formation and the mammalian lung produced by the budding process.

In an extensive study of the dried lungs of adult reptiles Milani, 94, 97, emphasizes the importance of septum formation for the differentiation of the pulmonary apparatus as one ascends the animal scale. The formation and enlargement of primary septa upon the dorsal and ventral walls of the lung cavity which extend horizontally from the median to the lateral wall of the lung as well as the further subdivision of these spaces by secondary septa is responsible for the gradual evolution of the complex from the simple lung.

Ever since the work of Kölliker, 79, the architecture of the mammalian lung has unanimously been conceeded by all who have worked upon the embryonic stages to rest upon a process of centrifugal budding. The centripetal formation of septa, apparently, plays no part in its evolution. There has been, therefore, a great gap between the developmental processes in the reptilian, amphibian, and avian lung, on the one hand, and the mammalian lung on the other, for, as Gegenbaur has pointed out, ontogeny and phylogeny have apparently stood in conflict, as the pulmonary apparatus in the ancestors of the mammals was produced by a process exactly opposite to that which ontogeny shows is responsible for the growth of the mammalian lung.

The first work which has offered us a suitable explanation of this

apparent discrepancy between the ontogeny and phylogeny of the mammalian lung is that of Moser, 00, who, in studying the comparative embryology of the respiratory apparatus in vertebrates, comes to the important conclusion that all vertebrate lungs are formed by a common growth process. In birds the respiratory apparatus is developed from a projection of the head gut, and its bronchial system results solely from a process of budding. In reptiles the growth process is exactly like that in birds, namely, by a bronchifugal system of sprouts while the septa are produced by relatively resistant points in the lung wall remaining between two of its outgrowing portions. This same method of growth, furthermore, is again repeated in a less localized and more diffuse form in amphibians where it gives rise, in the first place, to the dilated lung cavity, and, later, to the semispherical projections on the peripheral wall of the lung. In amphibia, as in reptilia, septa are formed by more resistant points in the lung wall remaining between two projecting portions.

Any doubts of Moser's method or results seem to be effectually silenced by the appearance of Hesser's, 05, careful and convincing paper on the development of the reptilian lung. Hesser finds the endodermal anlage of the reptilian lung appearing as a fold projecting from the head gut immediately behind the last gill pouch. This separates from the œsophagus in a caudocranial direction. From the cranial portion the trachea is formed, while the caudal part gives rise to the bronchi. The latter grow out as long, narrow tubes, at first in a dorsolateral direction, and, later, parallel to the median plane of the embryo. In the lizards, the bronchi begin to widen at the lateral side, making a sharp distinction between the extra-pulmonary bronchus and the future lungs. In species, however, where there is no extra-pulmonary bronchus, the dilatation affects the whole tube. We have then, at this stage, a respiratory anlage consisting of a long narrow trachea with two narrow bronchi arising from it. These terminate in two enlarged primitive lung sacs. At this point the inner surface of the lung becomes complicated by the more rapid growth of certain portions of the wall of the lungs by a hernia-like production of buds. This process begins in a cranial portion of the lung and proceeds gradually to its caudal extremity until finally a large number of buds surround the sac. In Tarentola, the most prominent group appears along the dorsal side of the stem bronchus, while the remaining sprouts occupy transverse rows alternating with the dorsal series.

While, in lizards, the stem bronchus is dilated, in turtles (with the exception of the caudal end which contains a large lumen) it remains a relatively small tube. The bronchi grow to considerable length before

branches appear. These are produced by buds or hernial projections from the wall of the bronchus. Upon the stem bronchus are produced, according to Hesser, a lateral and medial row of buds, a result in which he is not in accord with Moser, who believes that there are three series, a lateral, ventral, and dorsal. Especially noteworthy is the fact that in land turtles the lateral bronchi form dilated sacs which later grow into wide ducts, while in the sea turtles the buds grow out as small tubes somewhat dilated at the ends.

The question of the unequal development of the snake's lung has recently been taken up again by Schmalhausen, **05**, who finds in Tropidonotus natrix an unpaired pulmonary anlage. From its caudal end, appears later the two projections for the lungs, which grow unequally but continuously throughout embryonic life. The enormous overgrowth of the right lung leaves the left as a slight appendix upon it. There is, apparently, no regressive change such as Rathke supposes takes place in Coluber natrix. Schmalhausen's observations support Baumann's supposition on this point. More important, however, is a still further confirmation of the work of Moser and Hesser as the lung of Tripidonotus natrix grows not through the development of axipetal septa production but from an outward budding of the lung wall.

In view of these researches of Moser, Hesser, and Schmalhausen, then we may look upon respiratory apparatus of vertebrates as the resultant of a common principle of growth, and, in turning to the consideration of the ontogeny of the mammalian lung, there is good ground for believing that its developmental processes no longer conflict with its phylogeny. The evolution of the pulmonary system of mammals was first studied by Kölliker, **79**, who traced the development of the organ in rabbits. It appears from an unpaired anlage which arises behind the gill pouches. This is produced by longitudinal furrows which separate the head gut into a dorsal and ventral portion from the latter of which the lungs arise, while the former forms the œsophagus. On the tenth day, the lower part widens so that the lung anlage forms a half canal which ends caudalwards in two round depressions. Through a longitudinal fissure, the anlage is still in communication with the œsophagus, while both structures are surrounded by a mass of mesoderm. The projections forming the rudiments of the lungs grow rapidly and bend dorsalwards, and, at the same time, the trachea and œsophagus begin to separate. This process starts at the posterior end of the juncture and progresses towards the head.

A few years later Uskow, **83**, confirmed Kölliker's observations on the rabbit by finding on the tenth day evidences of separation of the

head gut into dorsal and ventral portions. From the ventral segment arises the respiratory system, while the dorsal is transformed into the œsophagus. About the level of the sinus venosus, the lungs appear as an unpaired dilatation of the ventral section and, synchronously, the trachea, also unpaired, is developed from the head gut just above it. Although the two structures appear simultaneously, the anlages, according to Uskow, are quite independent. Fol, 84, finds the origin of the lungs in a human embryo 5.6 mm. long as lateral diverticulæ on the head gut just behind the series of gill pouches. He is inclined to believe with Götte in the transformation of the last pair of gill pouches which have disappeared in the phylogeny of vertebrates into the respiratory apparatus.

His, 87, recognized the anlage of the human lung before the flexion of the embryo, that is to say, about the third week. It appears as a groove in the ventral part of the anterior segment of the intestine which becomes flattened just below the Fundus branchiales into a sagittal fissure and divides into an anterior and posterior half. From the former the trachea is formed, while the latter develops into the œsophagus. The respiratory portion begins above as a groove and ends below at the level of the auricles in a widened projection. From the latter, the lungs are evolved, while the former yields the trachea. At first, there is no medial division of the unpaired anlage which, save through the thickness of its epithelial lining, it is difficult to differentiate in the early stages. At the end of the first month the separation of the trachea from the œsophagus, beginning at the caudal extremity and proceeding upwards, is complete. And, as this separation takes place, there is a bilateral division of the anlage, which yields the primitive bronchi. These bend sharply dorsalwards, like a horseshoe, to embrace the œsophagus. The dilated primary lung sacs formed on these divisions are asymmetrical, the cause of which is probably to be sought in the first anlage of the lungs, which, according to His, does not show bilateral symmetry.

Up to this time Kölliker, Uskow, and His have agreed in their observations that the respiratory apparatus of mammals is derived from an unpaired anlage, but Willach, 88, in following the pulmonary system of the mole believes the trachea arises from an unpaired anlage, while the lungs originate as paired structures. The asymmetry of the anlage according to Willach is probably responsible for the greater development of the right over the left lung. In rats and mice, the process of development as described by Robinson, 89, agrees, in general, with the results obtained by His, Kölliker, and Uskow. Stoss, 92, and Bonnet, 92, in the study of sheep give results which accord with the findings of Uskow and Kölliker in rabbits, while Minot, 92, in his account of the evolution

of the pulmonary system in man, differs from His in looking upon the first anlage as symmetrical. Its subsequent asymmetry Minot believes is due to the unequal development of the heart.

In sheep, Nicholas and Dimitrova, **97**, find by the reconstruction method in an embryo of 5 mm. the main bronchi resulting not from a bifurcation of the primitive pulmonary projection, but as asymmetrical buds on its lateral face. Later stages, 7-9 mm., show an exaggeration of the precocious asymmetry as the right side is considerably more developed than the left, and the two primitive bronchi with the trachea form an inverted T.

Narath, **01**, followed the development of the lungs in rabbits and guinea pigs. In the latter, the development begins as a lateral flattening of the head gut just under the Fundus branchiales. This process continues until the lumen of the head gut forms a sagittal fissure just above the lower anlage, which, as it passes upwards, soon resumes its rhomboidal form. The ventral groove deepens and thickens, while, at the same time, the dorsal groove becomes narrower. Lungs and trachea arise from the ventral, while the dorsal part yields the œsophagus. Somewhat later a longitudinal furrow separates the two and the projection at the most caudal portion of the ventral groove, forming the first unpaired anlage of the lungs, shows a slight asymmetry as the right side is somewhat larger than the left. The lung anlage increases in size, ventrally, but even more markedly to the right and left. These two outgrowths, the anlage of the bronchi, show different relationships, as the right bends dorsally and caudally, while the left remains practically transverse. About this time begins the separation of the trachea from the œsophagus, which proceeds in a caudocephalic direction until the mesoderm surrounding the lung sacs not only projects into the cavity of the cœlom, but also passes in and separates the respiratory from the digestive portion of the head gut. The end of the lung sacs dilate, while still maintaining a marked asymmetry and, as this takes place, they extend dorsalward and embrace the œsophagus. In the development of the cat's and rabbit's lung, the transformation in general agrees with the conditions in the guinea pig so that Narath finds himself in accord with the earlier researches of Kölliker and Uskow, who also worked on the latter animal. Somewhat later Weber and Buvignier, **03**, in a comparative study of the origin of the lungs, especially in Minopterus Schreibersii, followed, by the reconstructive method, the lateral flattening of the post branchial region of the head gut. They describe a branchial crest, which descends from the last pair of gill pouches and terminates just before reaching the region in which the pulmonary appa-

ratus appears. The latter is formed from two asymmetrical thickenings of the lateral wall of the head gut, the left of which appears first in an embryo with 18 primitive vertebrae a little below and ventralwards to the last trace of the branchial crest. A constructive process, which these authors hypothecate, isolates the entire ventral segment of the head gut carrying with it the rudimentary lungs and extending as far cephalad as the last gill pouches. Weber and Buvignier obviously abandon the idea of the primitive unpaired anlage described by Kölliker, Uskow, and His, and with it the conception of a pulmonary groove formed syncronously with or before the lungs. Thus, the trachea is post-pulmonary in origin and is formed by this constructive process involving the ventral part of the head gut in the region behind the gill pouches. Like Götte in Anura, Kastschenko in the chick, and Fol in man, Weber and Buvignier look upon the pulmonary apparatus as diverticulæ of the head gut serially homologous with gill puoches.

Very briefly Blisnianskaja, 04, describes the anlage of the human lung as a projection in the ventral portion of the foregut, which, in an embryo of 4.5 mm., shows by two lateral grooves the beginning separation of the respiratory from the digestive system. At this stage, however, the two systems are still in open communication.

It is apparent that here is a practical unanimity of opinion among those who have contributed to our knowledge of the development of the mammalian lung as to the nature of the anlage and the process by which the primitive lung sacs are produced. Slight differences of opinion may be explained by the nature of the material and the methods by which the different observers have worked. Fol, who believes in a paired anlage for the human lung, studied an embryo somewhat older than the specimens of His and Blisnianskaja, while Weber and Buvignier and Willach, with this single exception, stand alone in regarding mammalian respiratory apparatus as arising from primitively paired structures. In turning, on the other hand, to the consideration of the organogenetic processes by which the bronchial tree is produced not only are few authors in accord, but, also, there is scarcely a chapter in the whole of embryology in which we find so many different opinions based apparently upon objective work. It will be wise, therefore, to consider briefly first the results which have been obtained by the different contributors to this chapter on the development of the lungs, and then attempt to make therefrom a fair statement of our knowledge of the architecture and origin of the bronchial tree at the present time.

Before the appearance of Aeby's paper we had no general conceptions concerning the architecture of the bronchial tree. According to the cur-

rent belief, as he himself points out, the division of the bronchi was dichotomous. Little of the origin, the relations, and mode of division of the bronchi was known and even less of the significance of the lobes either to each other or to the species in which they were found. Aeby, 80, graphically describes the darkness which surrounded our knowledge of the lung and blames the widely-accepted dogma of dichotomy for the condition. It is noteworthy, however, how the few objective investigators whose publications immediately preceded Aeby's also held his conception of the growth process of the tree. Among the first of these was Küttner, 76, who followed certain stages of the growth of the bronchi in the older stages of cow embryos and described the method of their proliferation as undivided from the end, that is to say, monopodial. From the stems of the bronchi, he says, lateral buds appear having their axes directed at right angles to the mother bronchus. By the subsequent rapid growth of these branches the monopodial character of the division is lost and an apparent dichotomy ensues. A year later Cadiat, 77, in sheep embryos measuring 12-15 mm. and upwards finds the trachea and main bronchi already well formed and describes the growth process as occurring not from the dilated ampullae at the end of the bronchi but rather from lateral outgrowths from their walls. In a slightly different way Stieda, 78, who also used sheep embryos supplemented by rabbits, came to practically the same conclusion.

In the year preceding Aeby's publication, Kölliker, 79, describes the appearance of secondary branches upon the primitive lung sacs in rabbits on the 12th day, when the stem bronchus of each lung has three projections. From this period the subdivisions become so numerous that it is difficult to follow them step by step, but, in general, the first branches pass dorsalwards and lateralwards. This branching, according to Kölliker, occurs from hollow buds or projections from the epithelial tube which multiply rapidly until each lung consists of a small tree of hollow canals with swollen terminal buds.

From these citations it is of course obvious that the idea of monopody was not new at the time Aeby wrote, and so the ignorance of the times concerning the *architecture* of the pulmonary tree was not, as Aeby supposed, so much due to the dogma of dichotomy as to the lack of a thorough piece of objective research such as he himself attempted to supply. And while many of his conclusions may find no place in our final conceptions concerning the structure of the lung, still they must always receive the credit of having furnished us with a working hypothesis by the aid of which the problem might be attacked by objective methods. His suggestive appeal to embryologists, of which His, 87, speaks later,

indicates his belief in the final solution of the question through embryological investigations. An interesting parallel, in a more limited way, might be drawn between the effects of Aeby's stimulating paper and the energetic investigations in the field of experimental biology which followed the annunciation of Weismann's views on heredity.

Aeby abandoned entirely any idea of dichotomy and substituted in its place a strict monopodial explanation of the arrangement of the branches of the bronchial tree. Each lung, according to this author, possesses a stem bronchus which forms its axis and leaves the lung at the hilum to fuse with its mate on the opposite side as they join the trachea. Of great importance is the relationship which the pulmonary vessels, especially the arteries, bear to the bronchial tree. The veins run in front of the bronchi, the arteries behind, as the latter are forced in leaving the heart to cross over the large air passages to reach their place. This crossing occurs near the upper end of the stem bronchus and divides the tree into two distinct segments of different importance. These are termed eparterial and hyparterial, according to their position with reference to the point where the pulmonary arteries cross the bronchi.

The arrangement of lateral bronchi is throughout typical and regular. Few occur in the eparterial while most are in the hyparterial zone. The former may be absent, but the latter are always present. The hyparterial systems of both lungs are symmetrical, but the eparterial systems, on the contrary, are ordinarily asymmetrical. The hyparterial bronchi always appear in two series, a dorsal and a ventral, which usually alternate and have their origin from the stem bronchus relatively close to each other, leaving the greater portion of the large bronchus free from branches. This forms then the angle of a three-sided prism from which the two series of lateral bronchi extend into the adjacent space bounded by the chest wall. The dorsal bronchi are shorter. The lateral bronchi give up some of their branches to the stem bronchus, a process which may be followed, according to Aeby, step by step, with the greatest clearness. These wander medialwards and finally cover the previously naked portion of the stem bronchus with dorsal and ventral accessory bronchi. These either remain close to the parent stem or else wander downwards. Their development begins usually quite far down the left lung, while in the right, they appear higher up and often produce a special bronchus supplying the Lobus infracardiacus known as the Bronchus cardiacus.

Eparterial bronchi are always single and never give off accessory branches. They arise from the stem bronchus at a point midway between the sites of origin of the lateral bronchi and divide generally into dorsal and ventral branches. One, especially the left, or both may be absent,

thus giving to us three principal forms to the bronchial tree, namely, (1) Lungs with an eparterial system on both sides; (2) Lungs with an eparterial system on the right side; (3) Lungs without an eparterial system. In some instances the eparterial bronchus is shifted back on to the trachea while in certain lower animals, especially the birds and reptiles, the eparterial system is more highly developed than in mammals. In the phylogeny of the lung, however, it becomes smaller until it may disappear entirely in some of the higher series.

In the further development of the lung sacs in the human embryo as described by His, **87**, all secondary bronchi arise from the first five primary divisions. Three of these occur on the right lung sac and two on the left. On the right side they are termed upper, middle, and end buds while those on the left are respectively lateral and end buds. With Aeby, His finds the primitive lungs prismatic in transection with one attached and two free angles between which lies its dorsal or costal surface. The stems give rise to the so-called ventral bronchi, which, His believes, should have been termed lateral bronchi. Owing, however, to the general acceptance of Aeby's nomenclature, he has followed it. From the stem bronchus dorsal branches appear which like the ventral group subdivide regularly. These secondary branches are accordingly designated as follows:

1. Bronchus dorsalis posterior.
2. Bronchus dorsalis lateralis.
3. Bronchus ventralis lateralis.
4. Bronchus ventralis anterior.

His agrees with Aeby with reference to the interpretation of the eparterial bronchus and looks upon it as an unpaired branch which, if it were in the hyparterial region, would divide into dorsal and ventral elements. As a matter of fact, after its appearance in the human embryo, it gives off branches which have these two general directions. On the other hand, he looks upon the Bronchus cardiacus as a true side bronchus, which, in opposition to the dorsal series, passes in a ventral direction. Its independence is shown in its early appearance as well as by the distance which separates it from the first and second ventral bronchi. It is regarded by His as an element which appears out of the schematic order and follows its own development. In the left lung, cardiac and eparterial bronchi are lacking, but the first ventral bronchus sends up a strong dorsal branch, which mounts up into the apical region of the left lung and is designated the Bronchus ascendens. In this way a substitution is made for the eparterial bronchus of the right side which, with the

absence of the Bronchus cardiacus, destroys the absolute symmetry of the hyparterial region. His followed the successive appearance of the chief bronchi and their main branches by the reconstructive method as far as embryos of the second month.

The growth of the tree occurs according to His by an extension of the root branches and a division of the end buds. In no place did he find evidences of lateral budding. The end buds during the process of division lose their conical form and flatten to some extent, while an elevation appears on one side which through the formation of a furrow leads to the outgrowth of two separate enlargements from the original bud. By the acquisition of cylindrical status on the part of these secondary buds the process can repeat itself. Below the region of the 3d hyparterial bronchus a point is reached where one cannot hold strictly to the principle of monopodial division, for it is impossible, His believes, to make as Aeby does the principles of monopodial and dichotomous division mutually exclusive. This, His remarks, is a conception of a somewhat transcendental nature, which leads the zealous investigator to personify his own ideas in the organ. The causes which control the form development of a growing tissue need not always remain the same, but may change its character once or several times. Accordingly, His summarizes the growth process from the unpaired anlage of the lung, which extends to either side in paired dilatations. From these primary sacs, lateral sprouts appear by monopodial growth. Further division is by dichotomy and finally a point is reached where the division occurs by more or less abundant lateral budding.

Willach, 88, studied several stages of the development of the lungs in the mole and pig, but his material, however, was not sufficient to give him a very complete picture of the gradual evolution of the pulmonary apparatus so he used the findings of other investigators to fill the gaps. Although Willach's own specimens did not include the stages of the first division of the primitive bronchi he believes the growth from first to last is monopodial, the end bud developing a lateral bud before its lumen narrows. These lateral buds become cylindrical as the parent bronchus continues to grow. Willach concludes from a study of the illustrations in His' paper that the eparterial bronchus is a derivation of the first ventral bronchus and looks upon it as an accessory branch in the sense of Aeby. He likewise believes that the apical branch of the 1st left ventral bronchus is analogous to the eparterial branch because, on its side, it bears the same relationship to the first lateral branch of the pulmonary artery that the eparterial does on the left. Willach follows the ideas of His in believing the Bronchus cardiacus is an independent lateral

bronchus and not an accessory bronchus in the sense of Aeby. In the case of the other so-called accessory bronchi, however, this author is in accordance with the views of the latter. Robinson, 89, studied the development of the lungs in rats and mice, and finds about the eighth day the primitive lung sacs growing lateralwards and dorsalwards, forming the bud-like projections into the cœlom from which the primitive and stem bronchi arise. The eparterial bronchus, according to Robinson, arises as the first division of the right lung bud. As a distinct branch, it is absent on the left side, although it is compensated for by a branch of the first lateral hyparterial bronchus, which is totally unrepresented on the right side and passes up to the apex of the lung. Robinson, in this view, is in accord with the findings of His. He believes the growth of the tree occurs by a flattening of the terminal bud opposite the axis of the bronchus and a subsequent division into two unequal segments of which the smaller becomes the lateral branch giving rise to what he terms an unequal or sympodial dichotomy. Robinson also describes branches arising as hollow buds from the main bronchus after it has resumed its cylindrical form, allowing the interpolation of secondary bronchi between those already existing, while the dorsal accessory bronchi of Aeby arise, according to Robinson, by a division of the primary dorsal bronchi, not by budding but by having the dorsal stalk split from the point of origin of the first median bud as far back as the stem bronchus, allowing this medial bronchus to obtain a secondary origin from the stem bronchus itself instead of from the primitive dorsal branch. The bronchus infracardiacus is ontogenetically a derivative of the main stem bronchus, but phylogenetically it is, as Aeby suggests, an original branch of the 1st hyparterial bronchus.

With the exception of the Bronchus cardiacus, Robinson has nothing to say concerning the ventro-accessory bronchi of Aeby. He calls them ventral bronchi, but it is not clear whether either ontogenetically or phylogenetically, as in the case of the most prominent one of the group, he considers them accessory branches of his lateral bronchi.

Ewart, 89, published a large monograph containing a criticism of Aeby's ideas on the architecture of the lungs. Ewart, like Aeby, used material consisting of dissections and corrosions of the adult lung, but only of one species, namely, man. Apparently this author did not perceive as clearly as Aeby that the hyparterial and eparterial theory was in reality a working hypothesis, which could only receive from embryological investigations the evidence necessary for its final substantiation or disproof. From his investigations Ewart believes that dichotomy, more or less equal, is the principle governing the division of the bronchi

from beginning to last. He abandons the distinction between the hyparterial and eparterial regions as well as Aeby's simple nomenclature and substitutes in its place a method of topographical designation which, besides going into endless detail, is constructed entirely independent of embryological considerations and has received, thus far, no support from subsequent investigators.

In a series of papers the first of which appeared the same year, Zumstein, **89, 91, 92, 00,** by the study of corrosion specimens of the lungs of a series of mammals and birds in which the pulmonary artery as well as the bronchial tree was injected is unable to support Aeby's conclusions with reference to the influence of the pulmonary artery on the architecture of the bronchial system. The division of the tree into eparterial and hyparterial bronchi according to Zumstein is not based on sound conclusions as he finds a series of variations in both arteries and bronchi, indicating that a formative influence in the sense of Aeby cannot exist. At the same time Zumstein studied the development of the lungs in the mole and the duck by the Born reconstruction method. With other investigators, he agrees in the precocious development of the right lung He does not describe in detail, however, the gradual evolution of the mammalian lung but simply states that the dorsal and medial bronchi arise later than the lateral branches but do not attain the extensive development of the latter. Whether or not he considers them accessory bronchi in the sense of Aeby is not clear from his description. The Bronchus infracardiacus may originate, according to Zumstein, either from the stem bronchus beneath the second lateral bronchus or from this bronchus itself. The eparterial branch of Aeby he designates as the first lateral bronchus. In the early stages the Arteriæ pulmonales originate far cranialwards and accompany the trachea ventro-lateralwards on both sides. The left is more dorsal even before the trachea is reached while the right artery passes ventralwards of the first lateral branch of the right bronchus (Aeby's eparterial). It is scarcely possible, Zumstein concludes, for the arteries to have an influence upon the structure of the tree as the first bronchi have appeared on the stem bronchus before the arteria pulmonalis can be traced into the lung.

In a preliminary note Narath, **92,** published a résumé of a large monograph upon the embryology and comparative anatomy of the bronchial tree of the mammalian lung, which appeared some nine years later, **01.** Before this work was published, however, Narath, **97,** described the development of the lung in Echidna aculeata. In all of the papers, he takes exception to Aeby's fundamental conception of the architecture of the bronchial tree. From a rich embryological material, echidna. rab-

bit, and guinea-pig, he describes the growth of the tree after the formation of the primitive lung sacs as taking place by monopodial growth with acropetal development of lateral twigs. In this process the stem bud is the principal structure, which grows on undivided with the ventral bronchi originating as lateral outgrowths upon it. The primitive lung sacs are to be looked upon, according to Narath, as the first stem buds. By this process arise from the stem bronchus two series of lateral branches, the ventral and dorsal bronchi. While the former are true derivatives of the stem bronchus, the latter, Narath is inclined to regard, as branches of the ventral bronchi which in course of ontogenetic and phylogenetic development are given up to the stem bronchus. From his embryological investigations, Narath supports Aeby's conclusions with reference to the dorsal and ventral accessory bronchi. They are formed first on the ventral and dorsal branches and then wander to their positions on the inner and ventral side of the stem bronchus. In this group and in complete accord with Aeby, he would also classify the Bronchus cardiacus except that, unlike Aeby, he believes it can arise in some instances from the second or third ventral bronchus. The pulmonary artery according to Narath's view has no great influence on the growth of the bronchial tree as he, like Zumstein, has found a whole series of variations in the artery without any important changes in the bronchi. Furthermore, he reiterates Zumstein's view that, both at the time the primary bronchi are formed, as well as later, the pulmonary arteries are thin, weak vessels of insufficient strength to influence these relatively thick and well-developed epithelial structures. Of equal importance in this connection is the observation that the arteries cross over the bronchi to pass down on its lateral, instead of its dorsal, side. Only at the end of the stem bronchus is its position distinctly dorsal. In consequence of this course, it forms a half spiral round the stem bronchus. Of a crossing in the sense of Aeby no true case exists. Narath accordingly proposes to abandon the distinction between the so-called eparterial and hyparterial regions of the bronchial tree.

The eparterial bronchus of Aeby has, according to Narath, the same area of distribution as a dorsal bronchus. He not only regards it such, but believes it is in reality, the first dorsal bronchus. To emphasize its special meaning for the topography of the lung, he terms it the apical bronchus. It is never suppressed nor does it degenerate in certain animals as Aeby suggests. It is, furthermore, always present normally as a lateral branch of the first ventral bronchus and possesses, moreover, the power of wandering up either onto the stem bronchus or the trachea. In speaking of his conviction that it is a real dorsal bronchus he con-

tinues: "Mit dieser einen Thatsache fällt die ganze Aeby'sche Theorie von den ep- und hyparteriellen Bronchien ein- für allemal." This view for which Narath has apparently received the entire credit in the literature was, as we have already seen, first announced by Willach. Narath's single addition to Willach's statement is in the designation of the eparterial branch as a dorsal element in conformity with his idea as to the possible derivation of the whole series of dorsal bronchi. In his belief, that the eparterial bronchus has the area of distribution of a dorsal bronchus, his observations are not in accord with those of Aeby, His, and Robinson.

Minot, 92, thinks the ideas of Aeby and His are erroneous with reference to the monopodial growth of the tree. He, on the other hand, looks upon the branching as characteristically dichotomous, describing the branches as having rounded ends. After division they develop unequally with the ventral fork, as a rule, serving as the stem. The first branches correspond to the lobes, but he does not agree with the findings of His and Aeby with reference to the presence of a bronchus in the right lung which is not represented in the left. With Willach and Narath he regards the eparterial bronchus of the right side and the apical branch of the first ventral on the left as homologous. The difference between the two, Minot holds, is due to the more precocious development of the right side and the secondary modifications in the arteries. The relationship of the veins confirms this view. The peculiar course of the right pulmonary artery is due to the abortion of the 5th arch on the right side and the subsequent transfer of the origin of the artery to the left.

In a series of papers d'Hardiviller, 96, 1, 2,; 97, 1, 2, 3, describes in the rabbit and sheep, the evolution of the tree after the trachea and main bronchi are laid down. There is, according to this author, a stem bronchus which transverses the whole lung and from which all of the primary bronchi are derived by means of collateral ramifications, that is to say, through epithelial herniæ from the walls of the stem bronchus, a process in which the terminal bud of the bronchus takes no part. In this way appear, in the rabbit, two buds on the right side and one on the left which, with the stem bronchi, enter into the formation of the five lobes of the lungs and produce all further ramifications. In the sheep, on the other hand, there are, including the stem bronchi, four buds on the right and two on the left giving rise to the six lobes of the sheep's lungs. The primary branches of the stem bronchus occur in four series, external, internal, anterior, and posterior, according to their position

on the stem bronchus. Of the four series, the primary, external, and posterior are the most important and are extensively developed, forming the principal bronchi of the adult lung. On the other hand, the anterior and internal proliferate to some extent but do not form extensively developed branches of the adult tree and are, therefore, termed by d'Hardiviller accessory bronchi using a similar nomenclature with a dissimilar meaning from Aeby and Narath. The further growth of the tree after the origin of the principal bronchi by collateral ramification, is by unequal dichotomy at first, and later, equal dichotomy. The processes differ with the different primary bronchi and appear earlier in the sheep than in the rabbit. The cardiac bronchus, according to d'Hardiviller, arises from the stem bronchus and, in this animal, remains independent. In the sheep, it emigrates on to the 1st lateral bronchus. The bronchus on the left side, he believes, always originates on the stem bronchus and wanders onto the 1st lateral thus forming the Bronchus cardiacus of Hasse. In the rabbit, d'Hardiviller finds the eparterial bronchus originating on the right side by collateral ramification, but unlike other investigators, he believes there is also an eparterial bronchus on the left. It appears on the 13th day and in 24 hours begins to degenerate and remains as a solid epithelial mass in connection with the mother bronchus. In consequence of his belief of the presence of this left eparterial element, d'Hardiviller thinks Aeby's classification of the lungs of mammals is only of secondary value. It also emphasizes its independent character and forces him to conclude that it is independent of Narath's apical bronchus as it is not a lateral branch of the first ventral bronchus.

d'Hardiviller's series of papers was interrupted by the appearance of a study by Nicholas and Dimatrova, 97, upon the development of the lungs in sheep by the Born reconstruction method in which they supported, in most respects, his observations. In an embryo of 5 mm. they find the main bronchi appearing as asymmetrical buds on the lateral faces of the anlage. In their later growth, this asymmetry is exaggerated. After the origin of the primitive pulmonary sacs two buds appear on their lateral walls (embryo 9 mm.) representing the first two lateral bronchi while simultaneously the tracheal bronchus is seen as an elongated projection on the right side of the trachea. No trace of a symmetrical bronchus, however, is found on the other side. They regard this element as being entirely independent of the bronchial system which must be regarded as a supernumerary bronchus originating from the future trachea just as the collateral bronchi are formed from the stems. The collateral bronchi, of which there are three sets, a lateral, a dorsal,

and a ventral, originate in the form of buds upon the bronchial stems. Each is an independent structure and does not show any ontogenetic relationship with the other bronchi, indicating a wandering of the accessory bronchial groups as described by Aeby, Willach, and Narath or d'Hardiviller, in the case of the cardiac bronchus of Hasse. From the division of the first lateral bronchi, a branch passes up towards the head on the left side which is unpaired, for on the opposite side this region is supplied by the tracheal bronchus. The infracardiac bronchus, Nicholas and Dimitrova regard as an unpaired precocious ventral branch for which there is no symmetrical structure in the left lung. The remaining ventral bronchi appear later as in an embryo of 18 mm. they find one between the second and third, and another between the third and fourth lateral element.

Huntington, 98, in studying the eparterial system of a series of adult mammals, comes to the conclusion that the right and left lungs agree morphologically in the type of their bronchial distribution and that the asymmetry is apparent and not real. These apparent differences are due to the shifting of a branch of the upper bronchus (cephalic trunk) which wanders up and becomes topographically eparterial. At times, the asymmetry may be more exaggerated by the migration of the entire branch. As the factor involved in this change is the bronchus itself and not the pulmonary artery, Huntington proposes to abandon Aeby's distinction between the hyparterial and eparterial regions of the bronchial tree except in a topographical sense. In the left lung there is a morphological equivalent for every eparterial element that may occur in the right lung and, accordingly, this author believes in the equivalent morphological value of the upper and middle lobes of the right side with the upper lobe on the left. This, it will be remembered is the conclusion of Willach and Narath except that Huntington, like Willach, does not believe that the eparterial element is primarily a dorsal bronchus. As the pulmonary artery does not run dorsal to the stem bronchus, but lateral, or dorsolateral, as Narath has shown, Huntington proposes to abandon also the distinction made by Aeby between the dorsal and ventral bronchi. From the study of his corrosions this author believes that the primitive type of division is practically dichotomous and later is changed into the monopodic system. Phylogenetically, the primitive type is the so-called bilateral hyparterial form, while the symmetrical eparterial type represents the end stage in the process of evolution and not the beginning as Aeby and Wiedersheim believe.

An ingenious effort is made by Guyesse, 98, to support the monopodial theory of growth. This author has studied the transformation of the

tracheal musculature into the muscle of Reisseissen in the successive branches of the bronchial tree. He finds the entire stem bronchus until it is past the divisions of the upper and middle lobe and projects well into the lower lobe has a musculature like the trachea. On the other hand, the bronchus of the upper, middle, and then lower part of the stem has the muscle of Reisseissen. These findings, Guyesse believes, give evidence that the production of the main bronchi is by monopodial growth.

Miller, 00, while working chiefly on the anatomy of the lobule, agrees, apparently, with Aeby's division of the eparterial and hyparterial region of the human lung, and, furthermore, he also speaks of monopodial division of the tree.

According to Justesen, 00, who studied the branching of the bronchial tree chiefly in cow embryos of well-advanced stages and in post-natal life, the division of the bronchi from first to last takes place by undoubted dichotomy after which the asymmetry is produced by unequal growth of the stem. This author approves of His' attitude towards Aeby's theory of monopodial development in general, but criticises his belief in the production of the first branches of the tree by monopody without having the material to follow their successive development. It seems rather strange, therefore, that Justesen, who was himself without these stages, should attempt to prove from His' illustrations in which these branches were already formed, that they originated by sympodial dichotomy especially after remarking so wisely, "Es ist kein Versuch, die Frage durch unberichtige Analogie folgerungen zu lösen. Ich will nur behaupten, dass die Frage nicht gelöst ist, weitere Untersuchungen dagagen nötig sind." Justesen does not believe in the production of bronchi by lateral outgrowths of the mother stem. He believes, therefore, Stieda's observation was faulty and states that no other investigator has since repeated this observation. He is ignorant, apparently, of the work of Robinson, d'Hardiviller, and Nicholas and Dimitrova.

Justesen does not accept Aeby's distinction between the eparterial and hyparterial regions of the bronchial tree and looks upon the accessory bronchi of Aeby as independent structures. Their irregularity he ascribes to the presence of the heart and vertebral column.

Merkel, 02, agrees with His, that the first divisions of the stem bronchi are produced by monopodial growth and that the later divisions arise by dichotomy. With Narath, he abandons Aeby's distinction between the eparterial and hyparterial region as resulting from the influence of the pulmonary artery on the architecture of the tree, and looks upon the right apical bronchus, the so-called eparterial, as a derivation of the first ventral and homologous with the apical branch of

the 1st ventral or lateral bronchus on the left side. Concerning the so-called accessory bronchi, Merkel seems to be in accord with the older observers in looking upon them as derivations of the dorsal and ventral lateral bronchi, and apparently follows Narath, instead of His, regarding the Bronchus infracardiacus as a possible derivative either of the first, second, or even third ventral bronchus instead of an independent branch of the stem.

The comparative embryology of the lungs in vertebrates has been studied by Moser, 00, whose material consisted chiefly of the lower vertebrates amplified to some extent by sections of rat, mouse, and rabbit embryos. All vertebral lungs, according to Moser, are developed through a common principle consisting in a general increase in size due to an increase of their constituent tissues. The epithelium is the principal factor which originates from the endoderm and passes as a single tube into a solid mass of connective tissue forming the framework of the lung. If this connective tissue is thin, the growth of the epithelium produces a widening of the intrapulmonary bronchus with simple projections on its walls as in amphibia. On the other hand, if the connective tissue is dense and resistant, the epithelial increase is localized in certain places, the cells are packed together until they force their way into the connective tissue forming buds such as we find in the lungs of all vertebrates from reptiles up. Certain points on the walls of the lung are more resistant and remain in the lung cavity as septa. At the same time, as we ascend the scale, the number of buds of the second order constantly increase. According to Moser, we may also observe at this time a gradual increase in the mass of connective tissue in passing from lower to higher vertebrates, and we obtain, in consequence, a system of long canals or bronchi passing through a connective tissue sac. The division of the bronchi is always and exclusively by monopodial growth, and is a main bronchus, the intrapulmonary bronchus, which is a direct continuation of the extrapulmonary bronchus passes through the lung from the root to its distal end.

By means of the reconstruction method, Bremer, 04, studied the lung of the young opossum (Didelphys virginiana) and compared it with older stages. His youngest specimen measured from 10.5 to 12.5 mm. and were taken from the same pouch. Older specimens, 14 cm. long, and three adults were also used for comparison. In five out of six of the new-born animals Bremer found an eparterial bronchus on both sides, except that the one on the left bronchus is always smaller and placed slightly lower than the eparterial branch on the right. The air chambers supplied by it, however, do not form the apex of the lung.

In spite of its small size and low position, it is above the first ventral bronchus and behind the artery and thus, according to Bremer, makes the right and left side of the lung symetrical and reptilian in type as no placentalian lungs are. The complete symmetry of the young lung is marred by the presence of a cardiac lobe on the right side which is unrepresented in the left. Bremer states that the reptilian lung has the double eparterial bronchus and thus the lung of the opossum is reptilian in type. In its later phases, the lung is changed from the reptilian to the mammalian form by the loss of the left eparterial bronchus, the multiplication of its bronchi and the acquisition of a new type of air chamber. In a 14 cm. opossum no trace of the left eparterial bronchus remains but Bremer states he is unable to follow the degeneration of this element from lack of necessary stages. He believes, however, with Selenka, that in the opossum we have an epitome of the evolution of the reptilian lung to the mammalian lung by means of the changes noted above.

The observations of Bremer at once recall the views of d'Hardiviller, who believes the left eparterial bronchus is always present in rabbits but subsequently degenerates. If this observation is confirmed it would seem to support d'Hardiviller's contention, although Narath, it will be remembered, believes that d'Hardiviller was dealing with a variation. From Bremer's statement that no other lungs of placentalia have the double eparterial system, it is apparent that he has overlooked Aeby's description of the lungs of Phoca vitulina, Bradypus tridactylus, Didelphinus delphis, Auchenia lama, Equus caballus, and Elephas Africanus, and some other nine species described by Narath and two species of Cebus by Huntington, making in all seventeen species where the condition described by Bremer as exceptional in mammalia is permanent. We must also consider the possibility that Bremer is dealing with a dorsal bronchus placed abnormally high on the stem bronchus, especially as he states this bronchus did not supply the apex of the lung. The observations of Narath, **96**, on Echidna aculeata are also suggestive in this connection as he states the relationships of the vessels, while young marsupialia are in the pouch suffer no further change either in the case of the arteries or the veins. Furthermore, Narath does not support Selenka, **87**, with whom Bremer is, more or less, in accord in his observations on the opossum lung as he finds the lung of Echidna develops like other mammalian lungs and is not differentiated from the developmental processes which are active in the production of the placentalian lung. He, therefore, does not approve of a comparison of the lung of marsupials with that of reptiles. Moreover, Hesser was unable to find an eparterial

bronchus or a bronchus which corresponded to it in his extensive work on the reptilian lung. (Personal communication.)

Blisnianskaja, 04, from the study of a series of models of the lungs of human embryos concludes that His' criticism of Aeby's nomenclature is correct, and accordingly divides the branches of the main bronchus into two groups, namely, a dorsolateral representing Aeby's dorsal series, and a ventrolateral including Aeby's ventral group. She states that this revision is justifiable even from a study of Aeby's own illustrations. These two series originate so that a line connecting their roots, from two more or less spiral lines on the stem bronchus. The eparterial bronchus, according to Blisnianskaja, is a dorsal branch of the first ventrolateral bronchus, which emancipates itself and wanders up on the stem bronchus according to the ideas of Willach, Minot, Narath, and Huntington. The entire dorsolateral group are similarly placed originally upon the ventrolateral group, they separate and wander up on the stem bronchus to receive a separate origin. As the eparterial on the right side is the first dorsolateral bronchus, Aeby's first dorsal bronchus becomes Blisnianskaja's second dorsal element. The apical bronchus on the left side is homologous then to the eparterial on the right side. The Bronchus cardiacus is also a division of the 1st ventrolateral bronchus on the right side, which separates from the mother branch, passes downwards, and receives a final origin upon the stem bronchus. Since the eparterial bronchus arises from the 1st ventrolateral, Blisnianskaja believes that the upper and middle lobe with the cardiac bronchus on the right side are equivalent to the upper lobe on the left side, and that the lower right lobe is equivalent to the left lower lobe. The form of the embryonic lung is influenced by the large fœtal heart and by the long development through which the human trunk, especially the thorax, passes. Blisnianskaja believes the method of division is sympodial or unequal dichotomy. She has never observed a bronchus originating from the complete bronchial tube by the monopodial growth.

A glance at this review of the literature shows a unanimous agreement among the various investigators only upon the independence of the lateral group of bronchi (ventral of Aeby, His, and Narath). There is, however, with the exception of Willach and Fol a general recognition of the fact that the mammalian lung arises from an unpaired anlage. Although supported by objective investigations, the interpretation of the origin of the other groups of bronchi, the method of their growth, and their significance for the architecture of the bronchial tree have varied within wide latitudes. We may be said at the present time to have no settled views upon the development of the bronchial system. In view of

the work of Moser and Hesser, the student of the mammalian lung, however, may look upon its phylogeny as being no longer in conflict with its ontogeny, and may also state his problem in the following series of questions:

1. Is the anlage of the lung unpaired or paired?

2. Is it symmetrical or asymmetrical?

3. Does the pulmonary artery exert any fundamental influence upon the growth of the bronchial tree, separating it into two regions of unequal significance as expressed in Aeby's Ep- and Hyparterial theory?

4. Is the "eparterial bronchus" an independent structure or a derivation of the 2d lateral bronchus? Is it an unpaired or paired element? Does an "eparterial bronchus" always form on the left side and then degenerate or undergo atrophic changes?

5. Is the Bronchus ascendens of His, or the left apical bronchus of Narath, the equivalent of the "eparterial bronchus"?

6. Are the lateral bronchi independent structures?

7. Are the dorsal bronchi independent structures or derived from the lateral group?

8. Are the ventral bronchi independent structures or derived from the lateral group?

9. Are the medial bronchi independent or derived from the dorsal group?

10. Is the Bronchus cardiacus an independent or accessory bronchus?

11. In what way do the bronchi grow? Does one system of growth predominate throughout the whole development of the bronchial tree?

12. What is equivalent value of the lobes of one lung in terms of the other?

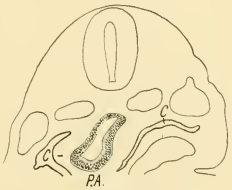
The Anlage of the Lungs.

The development of the respiratory apparatus begins in a pig by a lateral flattening of the head gut just below the Fundus branchiales. At the age represented by an embryo, 3.5 mm. nape breech measurement, the last gill pouch has in transsection (Fig. 1)* a flattened rhomboidal form with dorsal, ventral, and lateral angles. Below this gill pouch, lying behind the Sinus venosus, which already shows evidences of the increasing asymmetry of the heart, the ventral angle as it deepens to form the pulmonary groove (Pl. I, Fig. 1) is pushed somewhat to the right of the median plane (Fig. 1). The head gut in passing caudalwards,

* References to Text-Figures may read simply Fig. 1, or Fig. 2, or Fig. 3, etc., but every reference to figures on plates is accompanied by the proper plate number.

narrows gradually until its lumen in cross-section forms an asymmetrical sagitally placed fissure. A short distance above the Ductus hepaticus (Fig. 3 *DH*) the pulmonary groove terminates caudalwards in an irregular enlargement (Fig. 2 *PA*), the asymmetrical pulmonary projection forming the first unpaired anlage of the lungs. As yet, there is no trace of the main bronchi nor any evidence of a division. Ventralwards, it projects somewhat from the level of the ventral margin of the intestine below it (Pl. I, Fig. 1), while laterally it is more marked on the right than on the left side, an asymmetry more apparent from a transverse section (Fig. 2) or a dorsal view of the reconstructed intestine (Pl. I, Fig. 2). Whether the cause of this asymmetry lies primarily in the anlage itself or is due to the influence of the heart as Minot suggests, it is impossible to determine from these specimens. Below the pulmonary projection, the head gut while still asymmetrical lies more in coincidence with the median longitudinal plane.

Text Fig. 1.



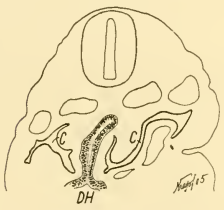
Text Fig. 2.

Text Fig. 1. Section of embryo pig 3.5 mm. long, showing head gut in the region of the upper part of the Mesocardium posterior. *C* = Cœlom. *SV* = Sinus venosus. *VM* = Mesocardium posterior.

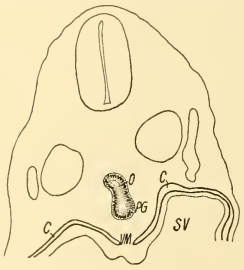
Text Fig. 2. Section of embryo pig 3.5 mm. long, through the pulmonary anlage. *C* = Cœlom. *PA* = Pulmonary anlage.

At this stage, the epithelial lining of the head gut is quite variable in thickness. In the pulmonary enlargement (Fig. 2 *PA*) it is clothed by a columnar epithelium of several layers with mitoses taking place chiefly in the innermost row. In the dorsal segment of the head gut at this level, it is considerably lower especially at the dorsal angle where it consists of a single layer. Above the projection it is thinner in the bottom of the groove and thicker at its sides. The Mesocardium posterior (Fig. 1 *VM*) begins just below the last gill pouch and extends down to a short distance below the pulmonary anlage. Between these points, the entire head gut is surrounded by a mesoderm composed of anastomosing cells in which the exoplasmic or fibrillar portion of the mesoderm is not well differentiated (compare Mall, 02, and chapter on

organogenesis). In the upper part of the gut just below the gill pouches, the mesoderm, covered by cœlomic epithelium forms slight asymmetrical projections into the cœlom (Fig. 1 *c*), while at the level of pulmonary swelling, the anlage of the mesodermic portion of the lung wings (Fig. 2) takes the form of two irregular lateral projections into the cœlomic cavity. The one on the right is much larger than that on the left (Fig. 2), so much so that at this stage the latter is only faintly shown. This results in a marked asymmetry of the primitive lung wings themselves. The mesoderm in the two wings is characterized by the richness of its cellular content, as the portion behind the intestine already shows a differentiation preceding the stages of chondrification of the primitive vertebræ. The mesoblastic anlage of the lungs arises from the general mesoderm of the head gut. Just below the pulmonary anlage



Text Fig. 3.



Text Fig. 4.

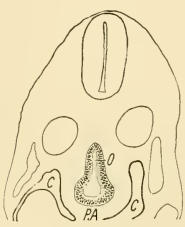
Text Fig. 3. Section of embryo pig 3.5 mm. long, through Ductus hepaticus. *C* = Cœlom. *DH* = Ductus hepaticus.

Text Fig. 4. Section of embryo pig 4 mm. long at the beginning of the Mesocardium posterior. *C* = Cœlom. *VM* = Mesocardium posterior. *SV* = Sinus venosus. *O* = Œsophageal portion of head gut. *PG* = Respiratory portion of the head gut.

on the left side are evidences of the Recessus pleuroperitonalis which, as described by Stoss, **92,** may at this stage be followed through a few sections.

In a slightly later stage, 4 mm., for example, the embryo shows the next step in the development of the respiratory apparatus. The head gut is more symmetrical with reference to the median longitudinal plane (Figs. 4, 5, 6). In the upper portion below the gill pouches, a longitudinal fissure appears on either side dividing it now into well-marked dorsal and ventral segments giving the gut in the respiratory level, more or less of an hour-glass appearance in transsections. These fissures mark

the line of separation between the respiratory (Figs. 4 *Pg*, 5 *PA*) and digestive systems (Figs. 4, 5 *O*) and extend from the region just below the gill pouches to the pulmonary anlage. In the upper portion, near the gill pouches, the lumen of the œsophageal part is somewhat larger, while, at the level of the pulmonary anlage, the respiratory segment is markedly dilated (Fig. 5 *PA*). Between these levels, the relationship between the two is practically equal (Fig. 4). Above, the epithelium is lower in the dorsal and ventral angles, slightly so in the lateral fissures but somewhat thickened at the sides of both dorsal and ventral segments. In these thickened portions there is a double layer, in the angles a single layer of epithelium. In passing caudalwards, the epithelium of the respiratory anlage thickens as its lumen increases in size until a double row of columnar cells line the floor of the pulmonary groove (Fig. 5 *PA*),



Text Fig. 5.

Text Fig. 6.

Text Fig. 5. Section of an embryo pig 4 mm. long, through the upper part of the pulmonary anlage. *C* = Cœlom. *PA* = Pulmonary anlage. *O* = Digestive portion of the head gut.

Text Fig. 6. Section of an embryo pig 4 mm. long through the lower portion of the pulmonary anlage. *C* = Cœlom. *O* = Digestive portion of the head gut. *BD* = Right stem bronchus.

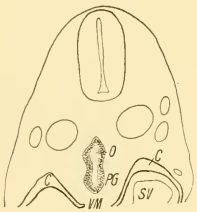
while at the sides, they are three cells deep. At the level of the pulmonary anlage, the asymmetry is again evident. The projection has now begun to extend lateralwards on each side to produce the main bronchi.

To the left, the evagination is considerably higher than on the right and also less prominent. At the same time the asymmetry is exaggerated by the anlage of the right bronchus (Fig. 6 *BD*) which points somewhat caudally. The epithelium lining the two primitive bronchi is columnar and consists of several layers. Rapid mitosis is taking place chiefly in the inner row of cells.

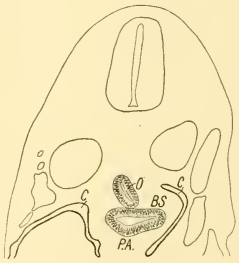
With the more marked symmetry of the head gut itself, there is also a greater symmetry of the mesodermal anlage (Figs 5, 6) of the lungs.

While the two wings still show the influence of the asymmetry of the bronchial projections, they are somewhat more regular than in the preceding stage. The anlage of the right wing is larger than the left and the Mesocardium posterior is also pushed slightly to the right. The character of the mesoderm remains about the same as in the last stage, that is to say, rich in cells with scarcely any differentiation of the exoplasm into primitive connective-tissue fibrils. Below the lung anlage, the Recessus pleuro-peritonealis is patent on the right side.

In a still later stage, 4.5 mm., the conditions remain practically as in an embryo of 4 mm. The most apparent differences lie in the further development of the two main bronchi. That on the left (Fig. 8 *BS*) grows practically at right angles to the axis of the pulmonary groove, while the right bronchus is directed laterally and caudally (Fig. 9 *BD*)



Text Fig. 7.



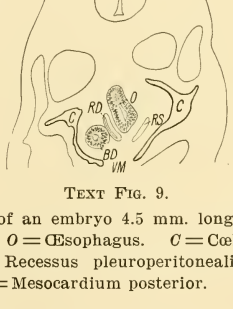
Text Fig. 8.

Text Fig. 7. Section of an embryo pig 4.5 mm. long at the beginning of the Mesocardium posterior. *C* = Cœlom. *PG* = Respiratory portion of the head gut. *O* = Digestive portion of the head gut. *SV* = Sinus venosus.

Text Fig. 8. Section of an embryo 4.5 mm. long, through the anlage of the stem bronchi. *C* = Cœlom. *PA* = Pulmonary anlage. *BS* = Left stem bronchus. *O* = Œsophagus.

and extends through a number of sections after the other has disappeared. From the anlage at the point of origin of the bronchi, there is a crest-like projection of the epithelial tube in the midline which is exaggerated by the slight dorsal flexure of the two main bronchi. This is scarcely seen in cross-sections, but can be made out easily in embryos cut longitudinally. At this stage, we also note the beginning of the process of separation of the respiratory from the digestive tract in a sulcus (Fig. 8) formed below the pulmonary anlage just behind it and in front of the ventral part of the œsophagus which is continuous above with the lateral fissures. In this particular embryo, the process seems a little

precocious as I possess later stages where the two systems are in open communication at a lower level than is shown in this specimen. At the level where the Mesocardium posterior begins (Fig. 7 *VM*), the epithelium lining the fore gut is columnar and consists, except in the ventral and dorsal angles, usually of a double layer of cells. In the anlage of the lungs (Fig. 8), it is slightly higher and shows a more active karyokinetic process. A similar layer of endoblast extends out into the primitive bronchi. At the tips, cell division is proceeding rapidly. The mesoderm of the lungs remains, so far as its differentiation is concerned, practically unchanged, but the lateral extension of the left bronchus now makes the projection into the cœlom at this level more marked than on the right side as the right bronchus, lying in a caudo-lateral direction nearer the median plane, does not carry the mesoderm quite so far into the right cœlomic cavity. On both sides, the Recessus pleuroperitonealis



Text Fig. 9.

Text Fig. 9. Section of an embryo 4.5 mm. long, through the lower part of pulmonary anlage. *O* = Œsophagus. *C* = Cœlom. *BD* = Right stem bronchus. *RD* = Right Recessus pleuroperitonealis. *RS* = Left Recessus pleuroperitonealis. *VM* = Mesocardium posterior.

may be seen. It is larger and extends higher on the right than on the left (Fig. 9 *RD, RS*). In Fig. 9, the beginning of the formation of the dorsal mesentery at the lower level of the lungs is apparent.

By the reconstruction process, the changes which have been occurring in the two preceding stages are demonstrated beautifully in a pig 5 mm. long where they are also considerably accentuated. Above (Pl. I, Figs. 3, 4) is seen a segment of the last gill pouch, while below it, the head gut narrows rapidly to a sagittal fissure forming the ventral respiratory and the dorsal digestive portion. The pulmonary groove, still in open communication with the œsophagus, terminates below in the asymmetrical

right and left bronchi. Of the two, the left (Pl. I, Fig. 3 *s*) passes lateralwards almost at right angles to the axis of the groove, while the right (Pl. I, Fig. 3 *d*) extends caudalwards and lateralwards, giving a sharp asymmetry to the fork which they form with the trachea (Pl. I, Fig. 3 *T*). From the slight crest in the midline which is not seen in the ventral view, both bronchi bend slightly dorsalwards. At the ends, there is a slight increase in the caliber of the bronchi, but end buds are not yet formed upon them. Underneath the point where the two unite, the sulcus from which the separation begins is already present, but it does not extend quite as far cranialwards as in the preceding stage. Viewed in profile, the whole anlage now extends somewhat ventralwards from the head gut, an extremely important relationship as we shall see in the chapter on the relation of the blood-vessels to the bronchial tree (cf. Schema A). The head gut below the origin of the two bronchi bends slightly ventralwards and to the left. In this region, which may be considered the anlage of the stomach, a noticeable dilatation of the gut is taking place (Pl. I, Figs. 3, 4).

In this stage the character of the mesoderm has not changed, the Mesocardium posterior begins at a lower level owing to the descent of the heart, while the dorsal mesentery is now well marked above the level of the lower extremity of the right bronchus. The two lung wings are more symmetrical and project further into the cœlom than in the preceding stage. Nevertheless, they are still asymmetrical in so far as the projection forming the left lung is higher than that of the right. Both on the right and left sides, the Recessus pleuroperitonealis is well marked. In another embryo of the same measurement, but evidently somewhat better developed, the process of separation of the bronchi from the œsophagus is well started. The sulcus between the trachea and the œsophagus extends just above the level of the origin of the two bronchi. This is filled with mesoderm of a nature similar to that about the head gut. The mechanical factors involved in the process are difficult to make out, but it begins by an approximation of the epithelium along the line of the two lateral fissures and then proceeds upwards from the sulcus formed behind the primitive bronchi which is filled with mesoderm.

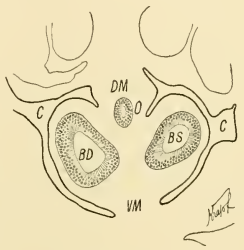
At this stage the following formula of the derivatives of the pulmonary anlage may be made:

TRACHEA.

Right bronchus. Left bronchus.

At 6 mm. (Pl. I, Figs. 5, 6) the process of separation is practically complete, the trachea and œsophagus remaining in communication only at the upper end. At the point of origin of the two bronchi, the œsa-

phagus and trachea are separated by a mass of mesoderm filling the intervening spaces. The simple bronchial system has increased in length and caliber, but the relationships are practically the same, save for the appearance of the rounded terminal buds on the end of the stem bronchi (Pl. I, Figs 5, 6 *d s*). While, in this embryo, the two bronchi still lie ventralwards to the head gut, they now begin at the ends to bend more dorsalwards than in the preceding stage, the right a trifle more than the left. The Mesocardium posterior is still lower than in the preceding stage, its upper level now beginning only a short distance above the origin of the left bronchus. The mesodermic syncytium is unchanged. The lung wings are fairly symmetrical as they project on either side into the cœlomic cavity. The difference, however, between the right and left lung bronchi still suffice to give the two lungs a slight asymmetry. The Recessus pleuroperitonealis is marked on the right side and ex-



Text Fig. 10.

Text Fig. 10. Section through the primitive lung sacs of an embryo 7.5 mm. long. *C* = Cœlom. *BD* = Right stem bronchus. *BS* = Left stem bronchus. *DM* = Dorsal mesentery. *VM* = Mesocardium posterior.

tends some distance above the lower end of the right bronchus, while the left recessus is almost obliterated.

The next stage in the development shown in an embryo 7.5 mm. long consists in the complete production of the primitive lung sacs through the dilatation of the buds on the end of the right and left bronchi (Pl. I, Figs. 7, 8 *s d*). The size of the branches of the primitive tree have increased markedly, the two dilated lung sacs while still lying ventralwards of the œsophagus now bend sharply backwards forming a horse-shoe-like curve around it (Fig. 10). The left still preserves its position at right angles to the trachea with a slight growth caudalwards at the bottom of the sac. On the right side, the general direction of the bronchus is lateralwards, dorsalwards, and caudalwards. The form of

the dilated sacs is different on the two sides, that on the right is larger and more nearly triangular in transsection (Fig. 10 *BD*). It projects further dorsalwards than the left (Fig. 10 *BS*). As yet there are no marked evidences of the production of lateral branches except a slightly more prominent angle at the upper lateral wall of the right sac and a similar irregularity of contour on the upper wall of the left. From these points, as we shall see in the next stage, the paired second lateral bronchi arise. Just above the origin of the stem bronchi, however, on the right side of the primitive trachea, one observes a slight bulging or outgrowth of its wall. At this level, the epithelium is a trifle thicker and numerous mitotic figures occur. The projection extends over an area of about 80 mikra and represents the anlage of the first lateral bronchus (Pl. I, Figs. 7, 8, L. 1). The process by which this structure is produced is apparently a simple evagination to be compared, perhaps, with the evagination of the pulmonary swelling from the primitive head gut, on the one hand, and the primitive bronchi from the pulmonary anlage on the other. Thus we may consider the same process as repeating itself in the development of the first stages of the pulmonary apparatus. No similar evagination, however, can be observed on the left side.

In the mesoderm of the lungs, the dorsal mesentery (Fig. 10 *DM*) now reaches as high as the forking of the trachea, while the Mesocardium posterior (Fig. 10 *VM*) extends as high as the anlage of the tracheal bronchus. The mesodermic syncytium itself shows some differentiation, particularly under the pleura and in the region of the mesocardium and dorsal mesentery. Here the cells branch and anastomose and the differentiation of the exoplasmic portion into fibrils is in progress. About the œsophagus and pulmonary epithelium, however, there are dense masses of mesodermal cells without much differentiation. This group of cells is engaged in the production of the young basement membranes as the stems continue in their growth. In consequence of the more equal dilatation of the sacs, the simple lung wings are more symmetrical than at any other period of early embryonic life. Differences, however, between the two sides on inspection of the reconstructions are readily made out. The right Recessus pleuroperitonealis extends slightly above the level of the lower end of the stem bronchus, while the left has disappeared. At this age we may express the derivations of the pulmonary anlage in the following tabulation:

TRACHEA.

Lateral 1.	
Right bronchus.	Left bronchus.
Right lung sac.	Left lung sac.

At 8.5 mm. the irregular contour of the lung sacs is lost and the two

bronchi continue their growth after the production of the first two paired lateral bronchi. These appear as lateral evaginations from the walls of the primitive sacs. On the left side, however, the bud is directed more cranialwards owing to the horizontal position of the left stem. The trachea increases in diameter and length; the bronchi, however, still maintaining the same general relationships, have grown in both caliber and thickness. Now, the very slight evagination of the tracheal bronchus has increased considerably in size and projects from the right wall so as to be noticeable particularly in longitudinal sections from which the model shown in Pl. I, Figs. 9 and 10, was reconstructed. It is quite as apparent as the paired Lateral 2 and is separated from the one on the right side by a distance of approximately 380 mikra. These three bronchi may be considered as practically contemporaneous branches of the primitive tree with the tracheal bronchus appearing as a very faint evagination before the lateral bronchi as such can be definitely seen in the primitive lung sacs.

The two stem bronchi now extend more caudalwards than in preceding stages; of the left particularly is this true. They also preserve, although not to such a marked extent, the horseshoe-like dorsal curvature observed in a pig 7.5 mm. long. On their lateral surfaces are two slight evaginations, the anlage of the second lateral bronchi (Pl. I, Figs. 9, 10, L. 2). Of these the right project lateralwards, while the left points upwards. These two projections do not appear from a terminal portion of the end bud, but from its lateral surface. They are, therefore, the productions of a monopodial growth The epithelial lining in these primitive buds is a trifle deeper than in the other parts of the tubes and in the inner row karyokinetic figures are more numerous than in the other parts of the respiratory endoderm. The mesoderm about the buds does not appear either thicker or thinner than that on other parts of the respiratory tube. It is impossible, therefore, that this tissue can exert any marked growth influence in the production of these lateral branches. Much more probable are the space relationships to which the tube adapts itself as, lateralwards in the cœlom, we have one point of least resistance, while caudalwards between the thorax wall and the liver, is another. The bending of the stomach anlage to the left (Pl. I, Figs. 9, 10) for a time may have some influence on the growth of the left bronchus holding it in its more horizontal position. From this point the consideration of the development of the mesodermic portion of the lungs will be discussed in a separate chapter.

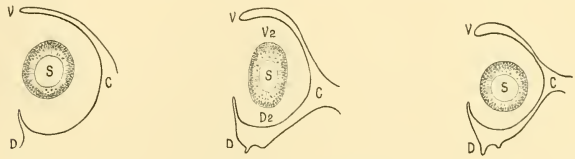
The branches of the primitive bronchial tree in a pig 8.5 mm. long, then, may be tabulated as follows:

Trachea.	
Lateral 1.	
Right stem bronchus.	Left stem bronchus.
Lateral 2.	Lateral 2.

At 10 mm., the trachea (Pl. I, Figs. 11, 12 *T*) has increased considerably in size and Lateral 1, which appeared as a simple swelling in the earlier stages, has now grown to a button-like enlargement (Pl. I, Figs. 11, 12, L. 1) sharply constricted from the wall of the trachea. It points lateralwards and also slightly ventralwards. The division of the trachea into right and left bronchi shows still the asymmetry of the preceding stages as the plane of the left stem is still more transverse than the right. At the same time the general direction of the right bronchus does not extend so far dorsalwards, as the growth of the right bronchus has apparently been directed more towards the tail end of the embryo. Just at the point of bifurcation, the second lateral bronchi on either side are seen; the one on the right is somewhat larger than the corresponding branch on the left. Both, however, are now fairly symmetrically placed, although right Lateral 2 is slightly more ventral and the left more apical in its direction. Beneath right L. 2, a slight bulging is visible on the axial bronchus directed ventralwards. This is the anlage of Ventral 2, the infracardiac bronchus (Pl. I, Fig. 11, V. 2) which arises directly from the stem bronchus and not from L. 2. At the same time, directly opposite the anlage of Ventral 2, there is also a slight dorsal evagination of the stem, indicating the first traces of Dorsal 2 (Pl. I, Fig. 12, D. 2) on the right side. The appearance of Ventral 2 (Bronchus infracardiacus) and Dorsal 2 is accompanied by an apparent lateral flattening of the stem bronchus due to the extension of the buds dorsalwards and ventralwards from the axis of the mother branch giving it, in cross-section, a marked oval shape, while above and below, it resumes its cylindrical form. This may be nicely seen in Figs. 11, 12, and 13, where 11 shows a transsection of the stem bronchus above, 13 below, and 12 at the level of the primitive dorsal and ventral branches (Fig. 12, V. 2, D. 2). In the inner row of epithelium in these projections, karyokinetic figures are much more numerous than in other parts of the stem bronchus save in the neighborhood of the terminal bud. At the same time there is a packing of the nuclei at the base nearer the basement membrane which is now less distinct and gives the epithelium the appearance of having an extra row of cells at this point.

The left bronchus is considerably shorter than the right and projects

more lateralwards. Its stem is cylindrical in form and it terminates in a rounded bud-like swelling in which mitoses are numerous. No evidences of Ventral 2 or Dorsal 2 are seen. If we turn for a moment to the consideration of the origin of L. 1, we find the bronchus is a trifle more precocious, but practically simultaneous with the second lateral branch in its origin. It is separated from Lateral 2 by a considerable distance. If the views of Willach and Narath were correct, this branch should not appear until later, and should be traceable, step by step, from the bud forming right Lateral 2 to its final position on the trachea. Its direction is practically lateralwards with a scarcely visible tendency to point ventralwards. It would not then, from the topography of its origin, bear any analogy to a dorsal bronchus. From this distinctly lateral position of its origin, I have classed it among the lateral group of bronchi, although, in its subsequent growth, one of its branches extends down into



Text Figs. 11, 12, and 13.

Text Figs. 11, 12, and 13. Sections through the right stem bronchus of an embryo 10 mm. long. Fig. 11 above, Fig. 12 through, Fig. 13 below the origin of Ventral 2 and Dorsal 2. *V* = Ventral. *D* = Dorsal. *C* = Pleural cavity. *S* = Stem bronchus. *V.* 2 = Ventral 2, the Bronchus cardiacus. *D.* 2 = Dorsal 2.

the dorsal region giving the bronchus a certain superficial resemblance to that series. On the other hand, the lower lateral elements grow ventralwards in the later embryonic stages and thus also lose their early strictly lateral character. This much is certain; if L. 1 arises phylogenetically from the dorsal group, a view for which there is no convincing proof, absolutely all trace of the migration is lost in the pig. It originates like one of the lateral bronchi and subsequently develops its superficial resemblance to the dorsal elements. Whatever support for the relationship of the bronchus to the dorsal series, can be drawn from this fact, is multiplied by the behavior of a lateral branch of left L. 2, which does exactly the same thing in an adaptative process on the part of the bronchus to a relatively unobstructed environment.

Similarly, Ventral 2 is produced after the formation of Lateral 2 simply as an evagination of the walls of the stem bronchus. It occurs at

a level below the point where the stem bronchus has already regained its cylindrical form after the production of the second lateral bronchus on the right side. Of the possibility of its being a branch of Lateral 2, in these specimens, there is not the slightest evidence. In this particular lung, D. 2 and V. 2 are given off at practically the same level. This is, however, not always the case as one, usually the ventral, may arise higher up. It is this variability in the origin of these branches which gives rise in the adult tree to the series of stages, which simulate a transplantation of the Ventral 2 from Lateral 2 to the stem bronchus. They represent, however, simply a normal range of variation in the origin of the bronchus. Narath states the wax-plate method is not adapted to the study of these branches and has, for the most part, used specimens cleared in oil of cloves. In my experience, the latter method is valuable only for the lateral bronchi where the buds are seen in profile and, therefore, are sharply outlined. In such specimens, either the dorsal or the ventral series must be studied not only through the mesoderm, but also through the entire thickness of the stem bronchus. In looking upon the surfaces of such buds as D. 2 and V. 2 in an embryo like that represented in Pl. I, Figs. 11 and 12, the slight projections forming the anlagen of these branches are invisible because they cannot be studied in contour. After they have developed into well-formed buds, they are quite apparent in cleared preparations, particularly when the stereoscopic microscope is used. By that time, however, the important stages of their origin are lost. So far as is known to me, reconstructions, controlled and supplemented by cleared and dissected specimens afford us the only opportunity to see the first traces of these branches. For such schematic pictures as shown by Narath, 96 (Text Figs. 1, 2, 3), which represent schemata of the origin of his apical bronchus and V. 2 from the bud of L. 2, I can find, in the pig, no parallel. Furthermore, the bud of V. 2 is shown in the schemata before the apical bronchus appears, while in the pig the latter is either the independent precursor or the contemporary of Lateral 2, while Ventral 2 is not formed until after the other two branches are well developed.

At this stage the following divisions have appeared in the primitive bronchial tree:

Trachea.

Lateral 1.	
Right stem bronchus.	Left stem bronchus.
L. 2.	L. 2.
V. 2.	
D. 2.	

In a reconstruction of the bronchial tree of a pig 12 mm. (Pl. I, Figs. 13, 14) the trachea and stem bronchi have increased considerably

in size. At the same time, Lateral 1, the tracheal bronchus (Pl. I, Figs. 13, 14, L. 1) has grown further lateralwards. Its terminal bud beyond the constriction near the point of origin bends somewhat ventralwards in conformation to the topography of the environment of the thoracic cavity at this level. Its general course after its origin is dorsalwards causing its lower extremity to overlap the upper part of L. 2 (Pl. I, Figs. 13, 14, L. 2). The asymmetrical characteristics of the two-stem bronchi are also maintained, the right extending lower and nearer the midline than the left, which projects more lateralwards. They also bend slightly dorsalwards. It is probable, however, that the asymmetry of this specimen is extreme, as I possess other specimens at this age in which the two sides, while markedly asymmetrical, are more nearly enanteomorphic than this one. In order to control this specimen, it was reconstructed a second time with exactly the same results. Of the two second lateral bronchi (Pl. I, Figs. 13, 14, L. 2), the right extends a little farther lateralwards and ventralwards than the left, its growth being influenced at this stage by the presence of L. 1 above and behind it. The left, however, with practically unobstructed environment grows lateralwards and dorsalwards and upwards at this period. Both are terminated by the end buds, which like that on L. 1, are in a stage preparatory to division. On the right side, Ventral 2 (Pl. I, Fig. 13, V. 2) the Bronchus infracardiacus has developed to a button-like bud on the ventral portion of the stem bronchus separated from it by a sharp constriction at the base. It is not so well developed as the two second lateral bronchi or L. 1. On the corresponding portion of the left stem bronchus, no analogous branch has appeared. It remains, in fact, naked through the whole future development of the tree. Neither is there in the pig, at this or later stages, a branch which forms at this point and subsequently wanders up on left L. 2, as d'Hardiviller suggests, to form the so-called cardiac bronchus of Hasse. On the lateral sides of both stem bronchi, buds forming Lateral 3 (Pl. I, Figs. 13, 14, L. 3) have appeared. These extend directly lateralwards for a short distance to terminate in swollen bud-like extremities, while the portion near the stem bronchus has a definite constriction. Of the two, the right is slightly larger than the left. From this point on, the stem bronchus continues caudalwards to terminate in the enlarged end buds. On the right side, the axial bronchus extends considerably lower than on the left. On the dorsal side of the stem between L. 2 and L. 3, appears on each side, the bud representing Dorsal 2 (Pl. I, Fig. 14, D. 2). That on the right side appears before the left and is a trifle more developed. The left, however, is quite apparent. It is also possible that either of these buds may not be

formed, in which case this area of the stem remains naked throughout life. This state of things, while occurring seldom, is found oftener on the right than on the left side and the cause may possibly be due, in this particular instance, to the presence of the rapidly growing Ventral 2, together with the presence of L. 1 above, or otherwise simply to the general tendency for the tree to vary within wide limits. As in the case of the ventral and lateral group, the position of these dorsal buds may vary from complete suppression to a position on the stem at the level of L. 2, or to one opposite Lateral 3. The usual situation is about midway between the second and third lateral branches. These buds are the same as Narath's Dorsal 2 and Aeby's Dorsal 1. Our results agree with Aeby's designation as Narath, in considering Lateral 1 and a dorsal branch, was forced accordingly, to change the denomination of his dorsal series. Like Ventral 2, I have designated the first dorsal bronchus as D. 2, simply to keep it in harmony with the lateral series.

At this period the following branches of the bronchial tree have developed:

TRACHEA.

L. 1.	
Right stem bronchus.	Left stem bronchus.
L. 2.	L. 2.
V. 2.	
D. 2.	D. 2.
L. 3.	L. 3.

13.5 mm. (Pl. II, Figs. 15, 16). At this stage the trachea is slightly larger and somewhat longer than in the preceding embryos. On its right side passing dorsolaterally is found L. 1 (Pl. II, Figs. 15, 16, L. 1) which has undergone division and yielded two practically equivalent branches, one of which passes downwards and dorsalwards (Pl. II, Figs. 15, 16 *di*) and the other lateralwards and slightly upwards (Pl. II, Figs. 15, 16 *vs*). These primary subdivisions, terminating in rounded buds, represent in the adult the dorsoinferior and the ventrosuperior branches of L. 1. At this stage, the two halves of the lung are much more symmetrical than we have seen them in any of the preceding reconstructions. The trachea and two main bronchi denuded of their side branches, now have more or less of a wish-bone shape. The trachea passes ventralwards to the origin of the stems and then, as the two axial bronchi diverge from the point of union, they also pass somewhat dorsally and reach their maximum point of separation at the level of the third lateral bronchi.. From this point, as the end buds are approached, they again converge towards the median line. The right is only slightly larger

and more developed than the left. At the same time, there has been a more symmetrical readjustment of the two second lateral bronchi, making them both with reference to their direction and the distance which separates them from the trachea practically mirror images of each other. L. 2 on the right side passes laterally and somewhat superior, undergoing like the tracheal bronchus a division into two practically dichotomous branches. Of these, one branch, which will continue as the main bronchus (Pl. II, Figs. 15, 16 *li*) lies ventralwards, while the other is directed dorsally and slightly inferior. The latter is the dorsal inferior branch (Pl. II, Figs. 15, 16 *di*) of the right L. 2 in the adult, and its downward course is due, as we shall see later, to the presence of L. 1 above, which prevents its growing upwards to the apex of the lung like the corresponding branch of the left side (Pl. II, Figs. 15, 16 *ap*). In comparing the growth of the three first divisions of the bronchial tree until they have reached their present development, it is possible to note in the progress of L. 1 and L. 2 on each side their passage through practically the same stages simultaneously. If the apical branch of L. 2 on the left side is equivalent of L. 1 or the tracheal bronchus as Willach, Narath, and others suggest, it is difficult to explain the tardy appearance of the left element and to give a reason why the right should be so well developed. As a matter of fact, this apical branch of the left Lateral 2 is not the homologue of L. 1, but of the dorsoinferior branch of right Lateral 2, a branch, which, in the adult lung, is practically but not quite as well developed as the apical branch itself. The difference between the two lies in the different nature of the environment in which they grow. Of equivalent age and value in the bronchial tree, the dorsoinferior branch on the right side, influenced by its space relationships and the presence of L. 1 above is forced to grow downwards and backwards, while on the left side, the corresponding branch, unobstructed through the absence of L. 1, mounts upwards to the apex of the lung to supply the territory through which the tracheal bronchus runs on the opposite side. This power of substitution, which the bronchi possess is not confined to this branch alone, but may take place in many other parts of the tree, as we shall see in the later stages. In my corrosions, I have never found an instance of the suppression of L. 1 in the pig. Narath, 01 (Pl. VII, Fig. 5), however, shows a case in the human lung which indicates how, under these circumstances, this dorsoinferior branch of right L. 2 with an unobstructed environment may take a course almost exactly like the corresponding branch on the opposite side.

Arising as in the preceding stages from the axial bronchus between L. 2 and L. 3, Ventral 2 on the right side has increased considerably in

length and passes ventralwards, medianwards, and caudalwards. At its terminus there is a definite bud. The corresponding portion of the stem bronchus on the left side, however, remains nude. In seeking for an explanation of the cause for the extreme development of Ventral 2 on the right side and its usual absence on the left, I have been impressed with the extreme adaptability of the lung to its environment and the way in which the bronchi follow mechanical principles in growing along the lines of least resistance. We realize, of course, the fact that the lungs are relatively late accessions to the animal economy, that they also, excepting possibly in marsupials, are functionless until the period of birth. It is natural, therefore, to find them secondary to and influenced by such organs as the heart and liver, as well as the chest wall by which they are surrounded. These, moreover, have chronologically the developmental precedence and are of definite functional use during the embryonic life of the organism. For the suppression of left V. 2 there is an explanation as we shall see in the chapter on the development of the pulmonary vessels and we may look upon the hyperdevelopment of right V. 2 as an effort to fill up the space which exists especially in quadrupeds between the heart and diaphragm in the region of the median plane. Dorsal 2 (Pl. II, Fig. 16, D. 2), situated between L. 2 and L. 3, shows a slight growth over the preceding stages, but still persists simply as a slight projection from the axial bronchus. The third lateral (Pl. II, Figs. 15, 16, L. 3) has increased in size over the corresponding branch in a younger embryo, and now possesses a more distinct terminal bud. There is, however, no indication of division as yet. On the ventral side of the axial bronchus, just beneath L. 3, there appears a slight swelling, indicating the origin of the third ventral bronchus (Pl. II, Fig. 15, V. 3). Directly behind it, on the dorsal surface of the stem, is a protuberance showing the point of origin of Dorsal 3 (Pl. II, Fig. 16, D. 3). Below these two branches, there is on the lateral side of the axial bronchus a bud indicating the point of origin of Lateral 4 (Pl. II, Figs. 15, 16, L. 4), while the axial bronchus continues downwards and ends in a terminal swelling on which some signs of the origin of L. 5 (Pl. II, Figs. 15, 16, L. 5) are already shown.

At this level, an evagination (Pl. II, Figs. 15, 16 *MS*) appears on the inner side of the end bud pointing medialwards and slightly dorsalwards just opposite the bud of Lateral 5. This is the first one of the medial series to appear on the reconstructions. They are, however, extremely variable both in their constancy and origin. In some trees they are entirely absent, in others they may occur with great regularity, but never in my specimens, which included sections and corrosions of over one hun-

dred lungs, do they occur higher than a short distance above the level of Lateral 4. They may exist only on one side or else on both. Like the other series, they arise as lateral outgrowths of the bronchial stem, not as secondary derivations of the dorsal series, according to the processes described by either Narath or Robinson. It is interesting, moreover, to note the relation of this group to the œsophagus. In the higher levels where the œsophagus lies between the stem bronchi, no medial bronchi occur, in the lower levels, however, as the œsophagus passes ventralwards to the stems, leaving the medial surfaces of the lung free, these branches are produced. Text Fig. 26 shows these conditions well. The edge of the œsophagus is seen in cross-section, while from the median wall of the end bud below it an evagination which will form a Medial 4 or 5 is clearly seen (Fig. 26 *M*). This would seem to indicate another adaptation on the part of the tree, to its space relationships.

On the left side, L. 2 (Pl. II, Figs. 15, 16, L. 2) is directed lateralwards and slightly dorsalwards. Like the corresponding bronchus on the right side, there has been a dichotomous division, which has yielded two branches, one directed dorsally and superior (Pl. II, Figs. 15, 16 *ap*) and the other lateral and ventral. The latter is the continuation of the main bronchus, while the former constitutes the apical branch, or Bronchus ascendens of His, of L. 2 on the left side. Owing to the unobstructed possibility of its growth upwards, inasmuch as there is on the left side no L. 1, this branch, as we have seen, grows in a slightly different direction from the corresponding division of the same lateral bronchus on the right side, but, for the reasons given above, it must be viewed as distinctly homologous with the dorsoinferior branch of right L. 2. This branch and its relationships may be seen in many of Narath's illustrations, from which the nature of its origin is as clearly shown as in the pig's lung. On the left side, there is no V. 2, but between L. 2 and L. 3, the Dorsal 2 (Pl. II, Fig. 16, D. 2), which already appeared in the earlier stages, is now well marked.

Lateral 3 (Pl. II, Figs. 15, 16, L. 3) is directed laterally and possesses a distinct bud at the end. It is also directed slightly dorsalwards, occupying a plane almost identical with the second lateral branch above. On the ventral surface of the axial bronchus, just below the point of origin of the third lateral, a small projection indicates V. 3 (Pl. II, Fig. 15, V. 3), while behind the stem bronchus, but somewhat lower, a similar projection marks the origin of the D. 3 (Pl. II, Fig. 16, D. 3). The fourth lateral bronchus (Pl. II, Figs. 15, 16, L. 4) exists at this stage

simply as a slight projection from the lateral wall of the axial bronchus as it continues downwards and ends in a terminal bud. The following is a tabulation of the tree in a pig of this age:

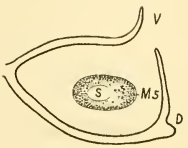
TRACHEA.

L. 1.	
(2) *DI.*	
(2) *VS.*	
Right bronchus.	Left bronchus.
L. 2.	L. 2.
(2) *DI.*	(2) *Ap.*
(2) *LI.*	(2) *LI.*
V. 2.	
D. 2.	D. 2.
L. 3.	L. 3.
V. 3.	V. 3.
D. 3.	D. 3.
L. 4.	L. 4.
L. 5.	
M. 5.	

In a pig 15 mm. long (Pl. II, Figs. 17 and 18) the trachea has increased in size and passes somewhat ventralwards to the point of bifurcation. On the right side and directed slightly dorsal and inferior, is Lateral 1 (Pl. II, Figs. 17 and 18, L. 1). A short distance from its point of origin, the ventral superior (Pl. II, Figs. 17, 18, *vs*) and dorsal inferior (Pl. II, Figs. 17, 18, *di*), branches are seen. These, in turn, now give rise to secondary branches. On the dorsal inferior branch, the first division (Pl. II, Fig. 18, *d*) is directed dorsally and somewhat medially This is the first main dorsal branch of the dorsoinferior in the adult lung, The other division continues on as the stem branch. The ventral superior bronchus passes laterally and superior. It now possesses a branch (Pl. II, Fig. 18, *d*) passing dorsally and slightly upwards. This is the dorsal branch of the ventrosuperior division of L. 1, and is found usually in the adult lung. The more general symmetry of the two main divisions of the trachea noted in the last reconstruction persists, the trachea passing downwards to the point of division and the right and left bronchi, as in the last stage, form with it a structure suggestive of a wish-bone. The axial bronchi bend laterally, dorsally, and medially, their point of widest divergence being now opposite the fourth lateral bronchi, a relation which persists in adult life, and with which the œsophagus, passing ventralwards at this level, probably has something to do. On either side, the second lateral bronchi pass lateralwards, then bend slightly dorsalwards and finally at their tips begin to bend ventralwards again. This indicates the

first appearance of the folding of the lung wings around the heart and liver, a process which is naturally directed largely by the form of the chest wall and shows another adaptation of the bronchi to the space in which they have to grow. As yet, however, the remaining lateral bronchi have not developed sufficiently to bend towards the ventral side of the body. On the right side, L. 2 has increased considerably in length, but possesses no more branches than the reconstruction of the preceding stage. The dorsal inferior branch, however, is considerably longer, and now grows distinctly downwards and lateralwards. Owing to the presence of Lateral 1, with the Lobus superior above and a consequent lack of space, this branch does not grow as rapidly as the relatively unobstructed corresponding branch on the left side, which, at this stage, is somewhat further advanced in its development. V. 2 (Bronchus infracardiacus) passes from its point of origin on the ventral side of the axial bronchus between L. 2 and L. 3, downwards, ventralwards, and medialwards. It is divided into branches of equal size, the first passing somewhat inferior (Pl. II, Fig. 17, *i*) and somewhat lateral, forms the inferior branch of the infracardiac bronchus in the adult. The other division passing more medialwards, is the continuation of the main bronchus. From the dorsal side of the stem, D. 2 (Pl. II, Fig. 18, D. 2) arises and subdivides into two short branches, the upper and median of which forms the median branch (Pl. II, Fig. 18 D. 2, *m*) of this trunk, while the other continues as the main bronchus. L. 3 (Pl. II, Figs. 17, 18 L. 3) passes lateralwards and slightly dorsalwards and, while considerably longer than in the preceding stages, it possesses as yet no secondary divisions. V. 3 on the right side is, in this specimen suppressed. It is noteworthy that next to Ventral 2 of the right side, this element of the ventral series is most often missing, a fact which may easily be accounted for by the hyperdevelopment of Ventral 2, which does not, as a rule, leave much territory in this region to be supplied by a ventral bronchus in this segment of the tree. Dorsal 3 (Pl. II, Fig. 18, D. 3) has grown considerably in size and now possesses a terminal bud. The fourth lateral (Pl. II, Figs. 17, 18, L. 4) shows a marked growth and is provided with an end bud, while between it and L. 5, on the ventral side of the axial bronchus a small projection indicates the fourth ventral bronchus (Pl. II, Figs. 17, V. 4). Immediately opposite it, D. 4 (Pl. II, Fig. 18, D. 4), arises as a small bud from the dorsal aspect of the axial bronchus, while Lateral 5 (Pl. II, Figs. 17, 18, L. 5) originates from the outer side of the stem as a small bud-like projection. From this point, the axial bronchus passes downwards and terminates in a slight end bud. On the left side, Lateral 2 shows a

marked growth of its apical branch (Pl. II, Figs. 17, 18, *ap*), which passes upwards and dorsalwards and terminates in two branches, one of which passes dorsally and inferior and indicates its first dorsal branch (Pl. II, Fig. 18, L. 2, *d*), while the other continues upwards as the extension of the stem of this bronchus. Near the extremity of L. 2 another branch is given off, which extends ventralwards and inferior (Pl. II, Fig. 17, L. 2, *vi*). This corresponds to the ventroinferior division of the bronchus in the adult lung. As there is no ventral bronchus between L. 2 and L. 3 on the left side, the axial bronchus remains at this point perfectly smooth. The second dorsal bronchus (Pl. II, Fig. 18, D. 2) of this embryo is placed somewhat lower than the corresponding branch of the opposite series and arises just above the point where Lateral 3 originates. Like its homologue, it shows a subdivision into two secondary branches, one of which is the regular medial branch (Pl. II, Fig. D. 2, *m*), while the other forms the stem of Dorsal 2.



Text Fig. 14.

Text Fig. 14. Section through the left lung of a pig 14.5 mm. long, showing the median evagination of the end bud to produce Medial 5. *V* = Ventral. *D* = Dorsal. *M* = Medial 5. *S* = lumen of end bud.

The third lateral bronchus (Pl. II, Figs. 17, 18, L. 3) grows lateralwards and dorsalwards, and is not provided with secondary branches at this stage. Appearing as a small bud from the ventral aspect of the axial bronchus, a short distance above L. 4 is Ventral 3 (Pl. II, Fig. 17, V. 3), while at a point about opposite this branch and a little above, Dorsal 3 (Pl. II, Fig. 18, D. 3) also originates as a small bud from the posterior surface of the stem bronchus, approximately midway between L. 3 and Lateral 4. The latter (Pl. II, Figs. 17, 18, L. 4) is somewhat shorter than the third, and has no secondary divisions. Ventral 4 (Pl. II, Fig. 17, V. 4) appears as a very faint swelling of the ventral aspect of the axial bronchus below L. 4. In a corresponding position on the opposite side of the main bronchus Dorsal 4 appears (Pl. II, Fig. 18, D. 4) also in the form of a slight evagination from the stem. Lateral 5 (Pl. II, Fig. 17, L. 5) is merely indicated by a slight swelling on the side of the

terminal bud of the axial bronchus. About opposite it on the inner side of the stem bronchus, is an evagination marking the anlage of a bronchus of the medial series (Pl. II, Figs. 17, 18, M. 5) like that seen on the right side at a similar point on the tree in the reconstruction of the preceding stage. Fig. 14 shows a section through the end bud where this element is in process of formation. The numerous karyokinetic figures and the definite extension of the evagination from the median portion of the lumen of the bud (Fig. 14, *S*) is clearly shown. This picture, when compared with the reconstruction and Text Fig. 26, indicates that there is no essential difference in the method of formation of these branches of the stem. Like the dorsal, ventral, and lateral elements, they are products of monopodial growth.

Following is a tabulation of the derivatives of the bronchial tree at this stage:

TRACHEA.

L. 1.
(2) *DI.*
(3) *D.*
(2) *VS.*
(3) *D.*

Right bronchus.	Left bronchus.
L. 2.	L. 2.
(2) *DI.*	(2) *Ap.*
	(3) *D.*
	(2) *VI.*
V. 2.	
(2) *I.*	
D. 2.	D. 2.
(2) *M.*	(2) *M.*
L. 3.	L. 3.
V. 3.	V. 3.
L. 4.	L. 4.
D. 4.	D. 4.
V. 4.	V. 4.
L. 5.	L. 5.
	M. 5.

In a pig 18.5 mm. long (Pl. II, Fig. 19, Pl. III, Fig. 20) the trachea is only a little larger than in the preceding embryo. It still passes slightly ventralwards from the upper end to the point of bifurcation. On the right side passing downwards and slightly dorsalwards, one finds Lateral 1 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 1), which divides almost at right angles into its main divisions, the dorsoinferior (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 1, *di*) and ventrosuperior (Pl. I, Figs. 19; Pl. III,

Fig. 20, L. 1, *vs*) branches. The dorsoinferior passes downwards and dorsalwards and terminates in the neighborhood of D. 2, a relationship which persists to the adult stage as its further growth downwards is now checked by the series of dorsal bronchi below. This branch shows new divisions over the preceding stage as we find besides the dorsal branch, which passes dorsalwards and medialward, a lateral branch (Pl. III, Fg. 20, *l*) arising about the same level, which passes laterally and dorsally. Both of these divisions terminate in end buds. The main stem of the bronchus continues downwards to its termination, which is marked by slight end swelling. The ventrosuperior or apical branch (Pl. II, Fig. 19; Pl. III, Fig. 20, *vs*) of L. 1, extends further cephalad than in the earlier stages. Besides the dorsal branch indicated in the preceding reconstruction, which shows signs of division, a lateroinferior branch (Pl. II, Fig. 19; Pl. III, Fig. 20, *li*) is given off somewhat further on, which passes at this time downwards and slightly outwards, and forms the first lateroinferior branch on this bronchus of the adult tree. The main stem continues upwards and ends in a terminal bud. The trachea and the stem bronchi still preserve the characteristic wish-bone appearance noted in the two preceding reconstructions. The two axial bronchi bending lateralwards, dorsalwards, and medialwards, the point of widest separation being, as in the earlier stages, about the level of the fourth lateral bronchi. In the preceding reconstruction, the beginning of the ventral growth of the two wings of the lung were apparent on Lateral 2. This action is now also shown on the third lateral branches. The first pair, however, curve around the heart, while those of the lower series follow the chest wall and the curvature of the diaphragm over the liver. The fourth, fifth, and sixth lateral divisions still pass outwards and slightly backwards without showing this bending at the extremities. On the right side, the second lateral bronchus arises about the point of bifurcation of the trachea, and passes slightly ventralwards, then runs upwards, slightly dorsalwards, and again ventralwards, preserving its course practically in one horizontal plane. In this specimen the first branch is a ventroinferior (Pl. II, Fig. 19, L. 2, *vi*), which extends downwards and ends in a bud, while the dorsoinferior branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2, *di*), which is scarcely larger than the preceding stage, is the second branch of Lateral 2. This condition indicates one of the very important factors in the growth of the bronchi, namely the ability of either branch after a division to continue on as a stem. In nine out of ten cases, the ventral fork, after the first division of Lateral 2, produces the main trunk, leaving the dorsal fork as the large

dorsoinferior branch, which is the equivalent of the apical branch on L. 2 of the left side. In this specimen, however, the ventral fork becomes the ventroinferior branch and the dorsal fork continues as the main bronchus, giving rise to the dorsoinferior branch only after undergoing another subdivision. In a much smaller percentage of lungs, the same thing happens on the left side, the ventral fork giving rise to a ventroinferior branch, while the dorsal grows on as the stem, producing the apical or stem only after passing through another division at the end. When this state of affairs occurs, we have the so-called "cardiac bronchus of Hasse," which d'Hardiviller believes is formed on the stem bronchus in the space for left V. 2, and then wanders up to Lateral 2. Of course in some animals Ventral 2 is formed regularly on the left side, and in others as a variation which establishes the symmetry of this segment of the tree. In the pig, however, owing to the relations of the pulmonary vein to this part of the stem (see chapter on pulmonary vessels), I have never seen a left Ventral 2. This power of the bronchi gives us a suggestive insight into the adaptations of the growing branches. The selection of the division to continue as the stem is probably governed largely by the physical environment in which the branches find themselves. As the conditions are usually the same, the same branches ordinarily become the stem, but if these are changed, what generally forms the stem is shunted off to become a side branch of relatively small size, while the division which usually constitutes the side branch, grows out as the stem and produces a numerous progeny of lateral divisions. In other words, the extent of the growth of a branch depends to some degree upon the nature of its physical environment. As I have stated above, owing to the generally fixed conditions, the major branches, especially such important ones at Lateral 2, have ordinarily a fixed type of division, but further out on the laterals or in the lower divisions, like Lateral 4 or 5 for example, this interchange of forks frequently takes place, as almost every specimen shows variations in the order of the branching.

The next division of the L. 2 is the ventrosuperior (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2, *s*), projecting from the main bronchus just external to the dorsoinferior branch, while a short distance lateralwards and dorsalwards is given off a dorsosuperior branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2, *ds*), which already shows indications of division. These branches represent apparently branches of the second order, but in reality, after a dichotomous division, each segment of the stem between the successive branches is equivalent in its order to that of the last lateral division. In the adult lung these branches are all easily recognizable.

Ventral 2, the infracardiac bronchus, has grown markedly, and presents a long inferior branch (Pl. II, Fig. 19, V. 2, *i*), which passes downwards and ventralwards and is indicated in the architectural history of the younger stages. The next division is a small bud from the upper portion of V. 2. (Pl. II, Fig. 19, V. 2, *vs*) which has a ventrosuperior direction and is found in specimens of the adult tree. This branch is small and at this stage consists simply of a slightly marked bud from the main bronchus. In most of the corrosions I have made of the lungs of older embryos it always shows by its flattened spreading branching that it is more or less influenced by the presence of the heart above it. The ventroinferior branch (Pl. II, Fig. 19, V. 2, *vi*), which is the next in order, is a slight bud, passing downwards and slightly ventralwards, and which, it may be worth while observing, with the inferior branch, sometimes substitutes for Ventral 3, when it is suppressed. After this branch, the main bronchus continues on to terminate in slight end swelling.

Here we are able to observe again the mechanical influence of environment on the growth of a bronchus. The inferior group of branches of Ventral 2 have space in which to grow and are accordingly of exaggerated size in comparison with the superior group, which cannot attain such extensive development, owing to the presence of the heart above them. In this bronchus, as well as in the laterals, we also have the possibility of propagation of the stem through either branch of a dichotomous division, as I have a number of specimens on which the ventrosuperior division arises before the inferior, indicating in these specimens, the use of the latter as the stem with the inferior branch arising from a subsequent forking. Right Dorsal 2 (Pl. III, Fig. 20, D. 2) of this specimen has not developed as far as the corresponding bronchus in the preceding stage, the terminal bud merely suggesting an approaching division, which was already well advanced in the bronchial tree from a 15 mm. pig. Such variations, however, are not uncommon. The third lateral bronchus (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 3) passes outwards and slightly ventralwards. From its dorsal aspect, a dorsal branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 3, *d*) originates, which terminates in the swelling already showing signs of division. The third ventral bronchus (Pl. II, Fig. 19, V. 3) arises from the ventral aspect of the stem, between Lateral 3 and 4 and grows downwards, apparently influenced by the marked development of Ventral 2 above it. Dorsal 3 (Pl. III, Fig. 20, D. 3), passes dorsalwards and lateralwards, and has a well-marked median branch (Pl. III, Fig. 20, D. 3, *m*) which terminates in a large bud, while the main bronchus points somewhat dorsally and laterally. Lateral 4 (Pl. II,

Fig. 19; Pl. III, Fig. 20, L. 4) has a definite ventral bud and at its ends is undergoing division. The fourth ventral bronchus (Pl. II, Fig. 19, V. 4) is somewhat smaller than the V. 3, and appears as a constricted button-like bud from the ventral aspect of the axial trunk, while Dorsal 4, arising at a somewhat higher level on the opposite side of the stem ends in a relatively large bud, which is as yet undivided. From the lateral aspect of the axial bronchus Lateral 5 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 5) takes origin, and ends in a terminal bud without division. D. 5 (Pl. III, Fig. 20, D. 5) is the smallest of the dorsal branches on this side, and appears simply a pedunculated projection from the dorsal aspect of the main stem, while the fifth ventral bronchus is present solely as a slight elevation or projection (Pl. II, Fig. 19, V. 5) from the ventral wall of the axial bronchus which, continuing caudalwards, ends in a terminal bud.

On the left side Lateral 2 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2), which was practically symmetrical with the corresponding branch on the right side in a pig 13.5 mm. long has now, in the rapid development of its main branch, lost even more than in the preceding stage its symmetrical relationships with right L. 2. The ventrosuperior or apical branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2, *ap*) is markedly increased in size, and now arises from the more superior aspect of the bronchus and passes superiorly and slightly dorsalwards. Its termination has reached a height equal to the point of origin of the tracheal bronchus on the right side. From its dorsal aspect, the first dorsal branch (Pl. III, Fig. 20, L. 2, *d*) is derived, which is now subdivided into two regular buds. A little higher, the lateral branch (Pl. III, Fig. 20, L. 2, *l*) is seen, while the apical end of the bronchus is in the stage of division. Further lateralwards, on L. 2 a dorsosuperior branch (Pl. III, Fig. 20, L. 2, *ds*) originates, which has a marked bud and is in process of division, while the next is an inferior or ventroinferior branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 2, *vi*) existing simply as a small pedunculated projection from the under surface of the bronchus. Lateral 2 terminates in a bud, which has undergone definite division, but the resulting branches are not yet sufficiently characteristic to be placed with reference to the adult tree. Inasmuch as Ventral 2 on the left side is always missing on the pig's lung, that aspect of the main bronchus remains perfectly smooth. At this period, however, the Vena pulmonalis already overlies this portion of the axial stem, but, for the sake of clearness in the illustration, it has been placed in approximately the median plane. Dorsal 2 (Pl. III, Fig. 20, D. 2) arising just above L. 3 passes dorsalwards, and has two marked

bud-like projections, one of which represents the median branch (Pl. III, Fig. 20, D. 2, *m*), usually the first branch of the dorsal series, which is already indicated in the preceding construction. Lateral 3 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 3) passes lateralwards and slightly ventralwards. It has a well-marked dorsal (Pl. III, Fig. 20, L. 3, *d*) and somewhat further out a ventrosuperior branch (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 3, *vs*), both of which are represented in the adult lung. The continuation of the bronchus ends in a bud, which is already undergoing further division. At a point just above the fourth lateral, Ventral 3 (Pl. II, Fig. 19, V. 3) arises, and ends in a slight terminal swelling. Dorsal 3 (Pl. III, Fig. 20, D. 3) is considerably smaller than D. 2, and also smaller than the corresponding branch on the opposite side, but is already divided into two buds, one of which represents the median branch of this bronchus, while the other forms the stem. Such variations in size as are shown in this instance, however, occur very frequently. Lateral 4 is somewhat shorter than L. 3, and has a well-marked ventral and a less marked dorsosuperior branch. The fourth ventral bronchus (Pl. II, Fig. 19, V. 4) is slightly smaller than the third and arises from the corresponding position in this interspace, while D. 4 (Pl. III, Fig. 20, D. 4) is considerably longer than the third, and ends in a bud which is not yet divided. Lateral 5 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 5) terminates in an undivided bud, and V. 5 (Pl. II, Fig. 19, V. 5) consists simply of a slight bulging of the epithelial wall of the axial bronchus. Similarly the fifth dorsal (Pl. III, Fig. 20, D. 5) is merely suggested by a faint projection from the epithelial tube. Lateral 6 (Pl. II, Fig. 19; Pl. III, Fig. 20, L. 6) is the smallest of the lateral series and ends in a slight swelling, while the axial bronchus continues downwards, terminating in an end bud. At this point the division of the stem is practically dichotomous. This specimen has no medial bronchi and is especially characterized by the lack of variations, for all of the bronchi, excepting the medial group, are present in almost schematic order. The entire absence of the medial group, however, must be regarded as exceptional for most trees, either on one side or both, have medial branches in some of the interspaces below the level of Lateral 4. While we have seen in the reconstructed series, examples of variations caused by the suppression of either a dorsal or ventral bronchus, another type occurs, not represented here, of which I have several specimens in my corrosions of the embryonic lung, namely, a reduplication of either the dorsal, ventral, or the medial bronchi in any one interspace. This may or may not be accompanied by a simultaneous suppression of one

of the adjacent elements of the same series. Following is a tabulation of the branches of a tree in an embryo 18.5 mm. long.

TRACHEA.

L. 1.
(2) *DI.*
(3) *D-L.*
(2) *VS.*
(3) *DS-LI.*

Right bronchus.	Left bronchus.
L. 2.	L. 2.
(2) *VI.*	
(2) *DI.*	(2) *Ap.*
	(3) *D-L.*
(2) *LI.*	(2) *LI.*
(3) *DI-S-DS.*	(3) *DS-VI.*
V. 2.	
(2) *I.*	
(2) *VS.*	
(2) *VI.*	
D. 2.	D. 2.
	(2) *M.*
L. 3.	L. 3.
(2) *D.*	(2) *D.*
	(2) *VS.*
	(2) *I.*
V. 3.	V. 3.
D. 3.	D. 3.
(2) *M.*	(2) *M.*
L. 4.	L. 4.
(2) *V.*	(2) *V.*
	(2) *DS.*
D. 4.	D. 4.
V. 4.	V. 4.
L. 5.	L. 5.
D. 5.	D. 5.
V. 5.	V. 5.
	L. 6.

Owing to the increasing complexity of the tree, it becomes almost impossible to reconstruct it by Born's method after this stage. At the same time I have not succeeded in getting good celluloid corrosions younger than 4 cm. pigs. This gap, however, has been partially bridged by drawings of the serial sections of the lung of a 23 mm. pig, aided by specimens cleared in oil of cloves, or injected and subsequently cleared according to the suggestion of Hochstetter, 98. By these methods, it is possible to follow the main divisions of the ramifications consider-

ably beyond that of the last reconstruction. With reference to the smaller buds, however, it is impossible either in sections or in clear specimens to determine definitely their course and final relationships. Nevertheless, as shown in these specimens, the bronchial tree evolves along the same lines. The tendency for the tips of the wings of the lungs to fold ventralwards around the heart and liver also becomes more exaggerated than in the case of the lung of a 18.5 mm. pig.

With the exception of the smaller buds, following is a tabulation of the main branches of the lung at this age.

TRACHEA.

L. 1.
(2) *DI.*
(3) *D-L-M-L.*
(2) *VS.*
(3) *DS-LI-LI-D.*

Right stem bronchus.	Left stem bronchus.
L. 2.	L. 2.
(2) *DI.*	(2) *Apical.*
(3) *D-I-D.*	(3) *D-L-M-D.*
(2) *LI.*	(2) *LI.*
(3) *VI-DS-I.*	(3) *DS-VI-DI-DS.*
V. 2.	
(2) *I.*	
(3) *DI.*	
(2) *VS.*	
(2) *VI.*	
D. 2.	D. 2.
(2) *M.*	(2) *M.*
(3) *S.*	
(2) *L.*	(2) *SD.*
(2) *M.*	(2) *L.*
L. 3.	L. 3.
(2) *V.*	(2) *D.*
(2) *D.*	(2) V.
(2) *SV.*	(2) *I.*
(2) *D.*	(2) *S.*
V. 3.	V. 3.
(2) *L.*	(2) *S.*
(2) *M.*	(2) *M.*
D. 3.	D. 3.
(2) *M.*	(2) *M.*
(2) *L.*	(2) *L.*
(2) M.	
L. 4.	L. 4.
(2) *V.*	(2) *D.*
	(2) *V.*

(2) *D.*	(2) *S.*
(2) *V.*	(2) *D.*
	(2) *V.*
D. 4.	D. 4.
(2) *M.*	*M.*
(2) *L.*	(2) *L.*
V. 4.	V. 4.
(2) *M.*	(2) *M.*
L. 5.	L. 5.
(2) *V.*	(2) *D.*
(2) *DS.*	(2) *V.*
(2) *I.*	(2) *DS.*
D. 5.	D. 5.
(2) *M.*	(2) *M.*
V. 5.	V. 5.
M. 5.	M. 5.
L. 6.	L. 6.
(2) *V.*	
(2) *D.*	
D. 6.	D. 6.
V. 6.	V. 6.

In a pig 5 cm. long, the bronchial tree can be studied by celluloid corrosions (Pl. IV, Fig. 21), but perfect specimens of the air passages in these small embryos are extremely difficult to obtain. The main features of the tree remain practically the same as in the earlier stages, save that it has increased markedly in the complexity of its branching. The trachea with its main bronchi maintains the wish-bone appearance observed in the reconstructions of younger embryos, but a marked difference is noted in the lateral bronchi, which now bend sharply ventralwards as the lung folds around the heart and liver, following the curve of the thoracic wall. The first lateral bronchus, while showing the chief characteristics observed in the younger stages, has a more complicated system of branches. It extends lateralwards and posterior, and divides into its two main branches, the dorsoinferior and ventrosuperior. The former runs dorsalwards, ventralwards, and posterior, while the latter branch passes anterior, ventralwards, and slightly medianwards. The main branches of the dorsoinferior bronchus are, at this stage, seven in number, and extend dorsally, laterally, and medially. Their serial arrangement may be determined from the tabulation at the end of this section. There are five main branches of the ventrosuperior or apical division, which have chiefly a dorsosuperior and a lateroinferior course.

Lateral 2 on the right side shows a marked increase in the complexity of its large dorsoinferior bronchus, which now shows six subdivisions.

The lateroinferior branch which serves as the continuation of the main bronchus, runs lateralwards, ventralwards, and slightly posterior. This has five main divisions, which have, in general, a ventroinferior and dorsosuperior course. V. 2, the Bronchus infracardiacus, passes medianwards, ventralwards, and slightly posterior. The main divisions noted in the earlier stages show an increase in their branching. Dorsal 2 extends in a dorsoposterior direction and its main branches radiate medialwards, lateralwards, and superior. The third lateral bronchus passes lateralwards, ventralwards, and slightly posterior. Its branches run ventrally, dorsally, and in a ventrosuperior direction. V. 3 bronchus in this specimens is not present. Dorsal 3 has four main branches, which have the same general direction as the second dorsal, namely, median, lateral, and superior. The fourth lateral bronchus has, at this stage, six main divisions, extending superiorly, laterally, and medially. D. 4 runs lateralwards, ventralwards, and slightly posterior, and has seven main branches passing in a ventral, dorsosuperior, and dorsoinferior direction. In this tree there is a median branch, M. 5, rising from the main bronchus opposite L. 5, the branches of which run in an ventrosuperior and a dorsoinferior direction. This bronchus is fairly constant, and is met with frequently in corrosions of older lungs. Its origin has been traced in the series of reconstructions of embryonic lungs from a medial evagination of the wall of the stem bronchus. D. 5 passes dorsalwards and slightly inferior. It has three main divisions extending medially, laterally, and inferior. The Ventral 5 runs ventralwards, medialwards, and slightly posterior, and has a medial and a lateral branch. Lateral 6 passes lateralwards, posteriorly, and to a slight degree ventralwards. It is, as yet, not long enough to show the ventral curvature, which is more marked in the lateral branches of the higher orders. Its branches, at this stage, run chiefly ventralwards and dorsalwards. Dorsal 6 projects dorsally and slightly posterior and has a single median division, while Ventral 6 as yet, has no branches.

Lateral 2 on the left side, owing to the further apical growth of its main division which passes up to the apex of the lung varies even more than in the preceding stage from the corresponding branch on the right side. This bronchus supplies the apical region of the left lung, which, in general, is taken by L. 1 and L. 2 on the opposite side, although the total volume of lung tissue is not nearly as great as that combined in the territory tributary to right L. 1 and L. 2. The apical branch grows almost directly superior, and has six main branches that run chiefly in dorsal, lateral, and medial directions. Its first main dorsal branch ex-

tends dorsalwards and slightly posterior, and bears a strong resemblance to the series of dorsal bronchi from the stem bronchus. Its branches run medially, laterally, and dorsoinferiorly. The continuation of the main bronchus, the laterointerior branch, corresponds in its course practically to the main branch of the opposite side. It possesses seven main divisions, which run dorsosuperiorly, ventroinferiorly, and dorsoinferiorly.

There is, as usual, no Ventral 2 on the left side. Lateral 3 runs laterally, ventrally, and slightly posterior. At this stage it has seven main branches, which pass dorsally, ventrally, superior, and inferior. While the remainder of the branches on the left side below this point show many asymmetrical arrangements from the corresponding divisions on the right, the architectural characters are sufficiently similar to avoid a repetition of the description. The main idea of these tabulations is to show the successive appearance of the chief bronchi of the adult lung and to indicate how the divisions are adapted to the space relationships to which the growing tree must adapt itself. It is not to be supposed that simple mechanical conditions govern entirely the growth of the bronchi, as its chief architectural features are undoubtedly phylogenetic. This much, however, is certain, that there remains always a considerable adaptability on the part of the growing branches, which is shown in their substitution power when one of the usual elements is suppressed, and apparently by the ability of either fork from a division to serve as the stem.

Following is a tabulation of the branches of the tree at this stage:

TRACHEA.

L. 1.
- (2) *DI.*
  - (3) *D-L-M-L-D-I-M.*
- (2) *VS.*
  - (3) *DS-LI-LI-DS-LI.*

L. 2.
- (2) *DI.*
  - (3) *D-I-D-V-DI-D.*
- (2) *LI.*
  - (3) *VI-DS-I-DS-VI.*

L. 2.
- (2) *Apical.*
  - (3) *D-L-D-M-V-D.*
- (2) *LI.*
  - (3) *DS-VI-DI-DS-VI-D-I-D-S.*

V. 2.
- (2) *I.*
  - (3) *DI.*
- (2) *VS.*
  - (3) *I.*
- (2) *VI.*
  - (3) *LI-VI.*

D. 2.
(2) *M.*
(3) *S.*
(2) *L.*
(3) *S-D-V.*
(2) *M.*
(2) *S.*

L. 3.
(2) *V.*
(2) *SV.*
(3) *DS-V.*
(2) *V.*
(2) *SV.*
(3) *D-V-S.*
(2) *V.*

V. 3 suppressed.

D. 3.
(2) *M.*
(2) *L.*
(2) *M.*
(2) *S.*

L. 4.
(2) *V.*
(3) *S-M.*
(2) *D.*
(3) *MS.*
(2) *V.*
(2) *VS.*
(3) *D-V-S.*
(2) *D.*
(2) *V.*

D. 4.
(2) *M.*
(2) *L.*
(2) *I.*
(2) *S.*

D. 2.
(2) *M.*
(3) *SL-IV.*
(2) *SL.*
(3) *S-LI-D.*
(2) *L.*
(2) *M.*
(2) *S.*

L. 3.
(2) *D.*
(3) *SM-IM-L-M.*
(2) *V.*
(3) *S-M.*
(2) *I.*
(3) *D.*
(2) *S.*
(3) *D.*
(2) *S.*
(3) *D-V-S.*
(2) *DI.*
(2) *V.*

V. 3. suppressed.

D. 3.
(2) *M.*
(2) *L.*
(2) *M.*

L. 4.
(2) *V.*
(3) *S-M.*
(2) *D.*
(3) *S-M-L.*
(2) *LS.*
(3) *D-V-D-V-S-I.*
(2) *D.*
(2) *V.*
(3) *M-L.*
(2) *S.*
(3) *V-D.*
(2) *V.*
(2) *D.*

D. 4.
(2) *M.*
(2) *L.*
(2) *I.*
(2) *S.*
(2) *M.*
(2) *L.*

V. 4.
(2) *S.*
(2) *L.*
(2) *M.*
(2) *L.*
(2) *M.*

L. 5.
(2) *V.*
(3) *S-M-L.*
(2) *DS.*
(3) *S-M.*
(2) *DI.*
(2) *V.*
(2) *S.*
(2) *DI.*
(2) *V.*

M. 5 opposite L. 5.

D. 5.
(2) *M.*
(2) *L.*
(2) *I.*

V. 5.
(2) *M.*
(2) *L.*

L. 6.
(2) *V.*
(2) *D.*
(2) *V.*
(2) *D.*

D. 6.
(2) *M.*

V. 6.

V. 4.
(2) *M.*
(2) *L.*
(2) *M.*
(2) *S.*

M. 4 between L. 4 and L. 5.

L. 5.
(2) *D.*
(3) *L-M-L.*
(2) *V.*
(3) *L-M-L.*
(2) *D.*
(2) *V.*
(2) *S.*
(2) *V.*

D. 5.
(2) *M.*
(2) *L.*
(2) *MS.*
(2) *L.*

V. 5.
(2) *M.*
(2) S.

L. 6.
(2) *D.*
(2) *V.*
(2) *I.*

D. 6.
(2) *M.*

V. 6.
(2) *L.*
(2) *M.*

In the study of the further development of the bronchial tree, I have made corrosions of the lung in a series of pig embryos of increasing age increments represented by a centimeter of growth up to and beyond the time of birth. From this series of corrosions it would be possible to tabulate the history of each bronchus until the full growth is attained. The results would be too detailed, however, to be of any value. Moreover, the wide range of variation of the branches destroys the absolute sequence of the branches in a successive series giving the formulæ only

an average relative value. Those which have preceded are, however, sufficiently constant to serve as a general guide to the direction taken by the main branches of the adult tree.

It may be well, however, to show pictorially the subsequent evolution of the tree without taking up the details of the branching, as a good corrosion of the bronchial system holds the general form of the lung quite as well as a hardened specimen of the lung itself. The tree of a pig 7 cm. long is shown in Pl. IV, Fig. 22. Besides the increasing complexity of the branching, one notes the ventral curvature of the lateral bronchi parallel with the chest wall. This is most marked in Lateral 2, less so as we proceed to Lateral 6. There are some peculiarities on this tree which are of great interest, for Ventral 3 on the left side is suppressed and in its place a prominent division of the second ventral or infracardiac branch has grown medianwards to take its place. A branch from Lateral 3 also runs to this region, giving an appearance as though it might be a ventral bronchus which had not left the lateral series. It is, however, a simple substitutive process on the part of the lateral branch for an element which has not developed in the earlier stages. This specimen also shows an instance where the dorsal fork of the first division of Lateral 1 continues as the stem, leaving the ventral fork, which usually serves that purpose, as a ventrosuperior branch, while the large dorsoinferior branch which is usually comparable to the apical branch on the opposite side rises from the next division. A median bronchus occurs on the left side opposite Lateral 4. On the right side, median divisions are not present.

In the corrosion of a tree from the lung of a pig 18 cm. long (Pl. IV, Figs. 23, 24) a number of interesting features may be observed, which serve to illustrate some of the developmental characteristics of the growing bronchial tubes. In the first place, we ordinarily have five paired lateral bronchi, while in this specimen there are but four. This indicates the suppression of the last of the lateral elements which is compensated for by an hyperdevelopment of Lateral 5 to supply the region usually tributary to Lateral 6. Accordingly the terminal forking of the stem bronchus, which usually occurs between Lateral 6 and the continuation of the stem, takes place in this instance between it and Lateral 5 (Pl. IV, Figs. 23, 24). While this tree shows the suppression of one of the lateral branches, I also have some specimens which present a series of six paired lateral bronchi below L. 1, indicating a possible variation in these elements between these limits with 5 as the average. Ventral 3 is suppressed on both sides, on the right it is

substituted for by inferior branches of Ventral 2 and partly by one of the branches of the first ventroinferior division of Lateral 4. On the left side, the ventroinferior divisions of Lateral 3 and Lateral 4 send branches to this region. Median 4 occurs on both sides opposite Lateral 4. It is particularly interesting to note the effect of the presence of median branches upon the dorsal series. Where median bronchi are present the median branches of the adjacent dorsal elements are very small and poorly developed, owing to the usurpation of their territory by this series. This naturally gives rise to the pictures which make it appear as though the median series might be transplanted elements from the dorsal bronchi. This relationship, however, is only another indication of the adaptability of the branches of the tree, for in this instance, had the median branches been suppressed, the median branches of the neighboring dorsal series would have grown over to occupy the territory in which the former are found.

In this specimen the ventral curvature of the lateral series is much more marked than in the preceding stage and now affects, to some extent, the whole lateral series, although Lateral 5 bends slightly, while Lateral 2 (Pl. IV, Fig. 23) shows an extreme ventral curvature, a characteristic which is progressively diminished until Lateral 4 is reached. This unequal bending has a marked effect on the stem bronchus and its other branches, and is responsible for the characteristic spiral-like insertion of the lateral and dorsal series upon the stem of adult lungs which has been observed but not explained by most of the investigators since Aeby. As the lateral bronchi turn ventrally more rapidly in the upper than in the lower series, the stem bronchus and its branches twist with them. Thus in the adult lung Lateral 2 appears to rise on the ventrolateral aspect of the stem and each successive element of the lateral series is inserted slightly more lateralwards. Similarly, on the adult tree, Dorsal 2 appears to originate somewhat on the dorsolateral surface of the stem, and the succeeding elements are successively inserted more directly dorsalwards. The spiral line connecting the origins of these two series of bronchi simply represent the degree of torsion of the stem bronchus as the lateral bronchi, in following the curvature of the chest wall, bend around the heart and liver. This is also nicely shown by the course of the pulmonary artery which, naturally, is mechanically influenced by the twisting of the stem bronchus as it is held in the angle formed between the lateral and dorsal series of bronchi. It is, of course, this secondary relationship of the lateral bronchi which led Aeby to term them ventral. In

their origin, however, they are, as we have seen, distinctly lateral, and I have applied to them, therefore, the genetic nomenclature.

The condition of the tree a few days after birth is shown in Pl. IV, Fig. 25. In order to show the three chief series of bronchi in a single illustration, Ventral 2, the Bronchus infracardiacus, has been broken off near the root. The tip of the ventrosuperior branch of the tracheal bronchus, owing to an accident, was also broken and should extend upwards and ventralwards for a considerable distance. Although the general form of the tree has not changed to any marked extent, besides the increase in the branching, the second laterals extend far ventralwards so as to embrace the heart. The effect of the presence of the heart on the tree, as in earlier stages, is shown particularly well by the direction of the branches of the tracheal and second lateral branches. The portions of these bronchi, which come in relation to the heart are nude, their branches extend so as to occupy the remainder of the chest cavity in their neighborhood, a relationship, which may also be seen by an inspection of the tables in the younger stages. Below Lateral 2, however, owing to a freer environment, the bronchi show the power of branching in any direction. In this specimen a few interesting variations are shown, one of which is of particular importance for comparison with the conditions shown in the preceding stage, namely, in the presence of seven lateral bronchi on the right side and five on the left. On the right side the whole ventral series is present, while on the left, two ventral bronchi occur between Lateral 5 and Lateral 6, a fact which would be difficult to explain if we viewed these branches as derivations of the lateral series since the entire group is complete from Ventral 3 down. Dorsal 3 on the right side is hyperdeveloped, while Dorsal 4 is quite small, a not unusual variation. None of my other specimens show such a marked development of the medial bronchi as Medial 4, 5, and 6, present on the right side, as well as an element of this series opposite Lateral 5 on the left side.

Relations of the Blood-Vessels to the Bronchial Tree.

In tracing the angiogenesis of the vascular system in the submaxillary gland and the suprarenal body, the author, **00, 02, 03,** showed that some of the mechanical principles, which Thoma, **93,** in his well-known researches found were involved in the development of the blood-vessels in the Area vasculosa of the chick, might be applied to vascular systems developing in three dimensions in the growing organs of mammals. Thoma found in the chick, that arteries and veins are originally simple capillaries. The subsequent transformation of the latter into arteries

on the one hand and veins on the other, is due to their fortuitous location with reference to the primitive aortæ and the venous ostia of the heart. Their growth in size bears a definite relationship to the velocity of the current in them, while their arterial or venous nature is determined by the character of that current, a high pressure pulsating column of blood giving rise to an artery, a low pressure constant current forming a vein. The nature of the current depends, naturally, mechanically upon its position on the arterial or venous side of the capillary plexus. In considering the problems of angiogenesis in mammals, I called attention to the fact that Thoma's principles do not explain all the facts of vascular development nor do they entirely accord with them. For example, the statement that a new growth of blood-vessels follows a rise of blood pressure in a capillary area must be considered only an hypothesis and not a demonstrated fact, for this would make the vascular system the stimulus for the new growth of cells, while it is much more probable that cells give the stimulus for the production of new capillaries. It is, of course, obvious that the principal factors that govern organic growth are resident in the cells rather than the blood-vessels as is indicated by their behavior in the embryo before the vascular system is laid down.

In tracing the development of the intrinsic vascular system of the mammalian lung, it is also obvious that the vessels follow the same histomechanical and histogenetic principles which are active in forming the vascular systems of such organs as the Gl. submaxillaris and the Gl. suprarenalis. Different conditions in the chief cells of the lung, namely, those of the bronchial tree, and different relations of the arterial supply and the venous drainage, give rise to different relationships on the part of the arteries and veins in the pulmonary apparatus. In the suprarenal body, we have the formation of a blood vascular system with a well-marked capsular plexus from which the blood supply of the organ is derived, and in the submaxillary gland an organ, where the blood-vessels, as in the lungs, accompany the ducts. In the latter instance, however, the conditions are such as to give rise to a venous system where the blood is drained by Venæ comites of the main arteries, while in the pulmonary circulation, a relationship exists in which the arteries and veins are separated from each other by means of the bronchial tubes.

According to the studies of Bremer, **02**, which have also been confirmed by Sakurai, **04**, the pulmonary arteries in the pig appear to originate symmetrically from the pulmonary arches like those of other mammals. At first they remain comparatively parallel and later (7-8 mm.) bend towards each other, sending out at the same time small branches which

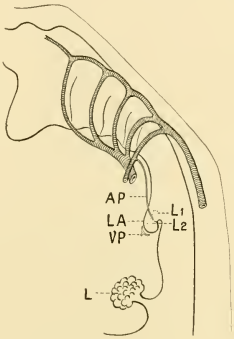
finally fuse into transverse anastomoses which yield ultimately a common trunk with two origins above and two main pulmonary arteries below. Bremer suggests that the bending of the arteries towards each other may be caused by the growth of the right and left auricles. This state of affairs occurs in the pig 11 mm. long. Later, the upper part of the right artery degenerates, and, with it, finally the right pulmonary arch. Thus we have the next stage where both arteries arise as a common trunk from the left pulmonary arch.

In the earlier pig's embryo (5 mm.), the arteries arising from the pulmonary arches on each side may be followed caudalwards a short distance from their origin on the arches, but only in particularly good specimens, as they are soon lost in the irregular capillary plexus surrounding the head gut to which, in their course, they give off frequent branches. At the same period, it is also possible to note the ingrowth of the pulmonary vein from the yet undivided portion of the auricle. It may be seen in a few sections running dorsalwards in the Mesocardium posterior towards the pulmonary anlage, which is, as yet, only partially separated from the œsophagus. It is asymmetrical as it lies slightly to the left of the medial plane. Its branches connect with the capillary plexus about the head gut and pulmonary anlage, establishing a venous outflow on the ventral side of the respiratory apparatus. Concerning the early appearance of the Vena pulmonalis in the pig, my observations are in accord with those of Narath on the rabbit for in these animals, the Vena pulmonalis is apparently evident at a much earlier stage than His, 87, or Schmidt, 70, were able to observe it in man.

At 6 mm. after the formation of the primitive lung sacs is well under way, the pulmonary arteries may be seen (Pl. I, Figs. 5, 6 *ad. as.*) running in approximately parallel courses until they diverge and are lost behind the right and left bronchi in the capillary plexus about the primitive lung sacs. Their course, however, on the two sides is different owing to the horizontal position of the left stem bronchus, the artery on that side (Pl. I, Fig. 5 *as*) is forced to turn dorsalwards in order to pass behind the left sac sooner than the right pulmonary artery, which maintains its more ventral course and, finally, at a lower level descends behind the right stem bronchus.

The factors which determine the course of the pulmonary artery in passing behind the lung sacs are, first of all, the ventral position of the venous outlet into the Sinus venosus, leaving the arteries to develop from behind. That is to say, with the increasing size of the right and left stem bronchi and the consequent enlargement of the capillary plexus

about them, it is natural, with the venous outlet already established on the ventral side of the sacs, that the capillaries on the dorsal side should enlarge into arteries. Furthermore, after its origin and partial separation from the œsophagus, the terminal part of the entire pulmonary apparatus extends somewhat ventralwards from the head gut making it additionally easier for the arteries to form on the dorsal than the ventral surface of the anlage. These factors are responsible for the course, which the arteries and veins take with reference to the bronchial tree, while the asymmetry of the stem bronchi appears to cause the chief difference in the course of the arteries on the two sides. It is, furthermore, possible that some of this irregularity is also due to the medial



Schema A.

Schema to show the origin of the relations of the pulmonary vessels to the lungs. *LA* = Lung anlage. *AP* = Arteria pulmonalis. *VP* = Vena pulmonalis. *L.* 1 = Site of origin of Lateral 1 the "eparterial bronchus." *L.* 2 = Site of origin of Lateral 2, the first bronchus in the "hyparterial region." *L* = Liver anlage.

bending of the right artery in preparation for its transfer from the right to the left pulmonary arch according to the suggestion of Bremer, although in Bremer's descriptions, with which my specimens agree, this actual transfer is made at a much later period, and I am accordingly inclined to minimize the possible influence of this factor. It is also worthy of note that we have no crossing of the bronchi by the arteries in the sense of Aeby. As they run down, they gradually turn dorsalwards to take up a position behind the primitive sacs and are lost in the capillary plexus, which surrounds them. The pulmonary vein, scarcely

longer than in the preceding stage, through the further growth of the auricular septum now empties into the left auricle.

In a pig 7.5 mm., the arteries (Pl. I, Figs. 7, 8 *ad. as.*) maintain the same relationship as those in the preceding stage, namely, the right lies more ventral than the left and also somewhat nearer the median line. Behind it, however, the evagination for the formation of Lateral 1 has appeared. At this time, the artery consists simply of an endothelial wall supported by the surrounding mesoderm. Situated some distance from the trachea, it is absolutely impossible that such a structure should have a determining influence upon either the production or position of this or other branches of the bronchial tree. Furthermore, it is now well known that such vessels do not influence mechanically the growth of organs which they supply, but follow the developmental processes which are inaugurated in the chief cells of the organ itself acording to definite histodynamic and histomechanical principles.

By a glance at the schema which elucidates this point, we see how the two factors outlined above have worked to bring about the relationship of the artery to the primitive lung sacs. After its origin during the production of the primitive lung sacs, the lung anlage (Schema *LA*) extends ventralwards. The Vena pulmonalis (Schema *VP*) in growing in from the auricle has established the venous outflow ventral to the anlage, leaving the pulmonary arteries (Schema *AP*) to form on the dorsal side of the primitive stems. This relationship occurs, however, before there is the slightest indication of the presence of any of the main bronchi. Later as they appear, Lateral 1, the so-called "eparterial bronchus" (Schema L. 1) develops behind the artery and Lateral 2 (Schema L. 2) in front of it. Sometimes Lateral 1 is higher up, where it appears on the trachea, sometimes lower down where it forms on the stem, often where it forms on both sides, the left is lower than the right. The most important element in determining the position of Lateral 1 is the point at which the trachea separates into the two stems. As we have seen, when this is high, taking Lateral 2 on each side as the fixed topographical point, Lateral 1 is on the stem; when it is low, as in the pig, Lateral 1 forms on the trachea.

It is also important to observe that the relationship between the Arteria pulmonalis and Lateral 2 is not "eparterial" as Aeby suggests; the artery in the embryo simply runs ventralwards to Lateral 1 and then passes gradually behind the stem. The "eparterial and hyparterial" topography of the bronchi is due to the descent of the heart in the later stages of embryonic life and to the degeneration of the Ductus arteriosus after birth when the entire circulation from the right ventricle, conse-

quently, is transferred from the systemic into the pulmonary system. Until this occurs, the pulmonary arteries do not even approximately cross the stem bronchi as Aeby suggests. Apparently, as we shall see later, he recognized this fact. Furthermore, my observations in older stages are in accord with the findings of Zumstein and Narath, who hold that, in the sense of Aeby, a true crossing on the part of the artery never exists. It seems to me important, therefore, for a logical conception of the architecture of the bronchial tree, that the terms "eparterial and hyparterial" or, at least, all that they imply should be abandoned.

The pulmonary vein (Pl. I, Fig. 7 *v*) is seen at this stage with two small tributaries, one from the head and another from the caudal region running in the Mesocardium posterior. They are in connection with other dilated capillaries which may be seen in the neighborhood of the lung sacs, but the latter have not become large enough as yet to form definite veins. The vascular apparatus of the lungs, then, at the period of the formation of the two lung sacs, consists in two small asymmetrical arteries passing down behind the primitive stem bronchi ending in an irregular capillary plexus about the dilated epithelial tubes from the ventral side of which run enlarged capillaries emptying into the pulmonary vein in the Mesocardium posterior.

No particular change is observed in the next older embryo 8.5 mm. in the relationships of the arteries (Pl. I, Figs. 9, 10 *ad. as.*). With the lengthening of the stem bronchi, however, owing to the increased capillary field about the bronchi, the right and left pulmonary veins (Pl. I, Fig. 9 *v*) may be seen emptying into the common trunk which, in turn, now opens into the left auricle. In a pig 10 m. long, the pulmonary arteries maintain their general relationship to the trachea, the right passing ventral to Lateral 1 (Pl. I, Figs. 11, 12 *ad*). Continuing downwards, they gradually extend behind the stem bronchi giving off branches to the irregular capillary plexus which surrounds the primitive tree, elements of which may be seen, here and there, in well-prepared cross-sections of the lung. As a rule, the arteries lie on the dorsolateral aspect of the stem. At this stage, it is quite evident that the three first branches of the tree, practically in the same period of development, are growing without reference to the arteries as they are surrounded only by a capillary plexus derived from branches of the arteries and from which dilated capillaries empty into the veins. As they increase in size, the arteries and veins, which follow the various ramifications of the tree are formed from the capillary plexus according to the regular histomechanical laws. The two main tributaries of the vein (Pl. I, Fig. 11 *v*)

forming the right and left stem veins, run on the ventromedial aspect of the stem originating from the plexus about the main bronchi. In this way, we have established the regular alternation of artery, bronchus, and vein which persists throughout the life of the tree, although it will be remembered that this relationship is due primarily to the position of the vein with reference to the anlage.

At 12 mm. (Pl. I, Figs. 13, 14) the vessels have followed the natural growth of the bronchi. From the capillary plexus on the dorsal surface of Lateral 2 on each side, the artery to that branch is formed. The vein (Pl. I, Fig. 13) by the rapid development of Ventral 2 is pushed somewhat medialwards at this point. With the marked development of Lateral 1, the tracheal bronchus, in a pig 13.5 mm. long, a branch (Pl. II, Fig. 15) is given off from the right pulmonary artery, which runs up along the ventral surface of the bronchus to end in the plexus about that branch. Continuing downwards, the arteries (Pl. II, Fig. 16) on both sides run on the dorsolateral aspect of the stem. The branches to Lateral 2 have increased somewhat in length, and from the right pulmonary artery a new branch is formed, which, passing under the root of right Lateral 2, ends on the lateral and under aspect of Ventral 2, the Bronchus infracardiacus. The artery still maintains its position with reference to the stem, which causes it to lie in the angle between the lateral and dorsal bronchi. Thus, the artery itself, however, is not responsible for the division of these two groups from the stem as Aeby implies when he says in speaking of Lateral 1, "In ihm hat offenbar die Scheidung des hyparteriellen Gebietes in zwei streng geschiedene Bezirke noch nicht stattgefunden, ein Thatbestand, der wohl damit in Verbindung gebracht werden darf, dass die Lungenarterie nicht sondernd einzugreifen vermöcht hat." Should we still suspect a causal relationship here, it is only necessary to glance at the ventral bronchi, particularly Ventral 2, to see an element not only originating from the stem away from the influence of the artery but also with its growth, developing from its capillary plexus an artery which passes around the stem and rests on its lateral side. Interesting changes, at the same time, are occurring in the veins (Pl. II, Fig. 15). From the tracheal bronchus, a branch may be observed passing down to the common pulmonary vein running still more ventral than the artery to Lateral 1, another one of the final adult relationships in the pig's lung. Here, however, we have an exception to the general relationships of the vessels to the bronchi due to the more ventral position of the veins and the failure of right pulmonary artery to form behind Lateral 1, which, in this particular instance, gives us a Vena comes to the artery to the tracheal bronchus instead of the usual alternation found in other portions

of the tree. On the ventral surface of Lateral 2, veins originate, which empty into the right and left pulmonary veins, while medialward and above Ventral 2 lies the vein of that bronchus which joins the right pulmonary just below the tributary from Lateral 2. In this stage, either owing to the hyperdevelopment of Ventral 2, or the increasing asymmetry of the heart, or both, the pulmonary veins are shifted somewhat to the left, causing them to lie somewhat beyond the median line. At the same time, the veins in these young stages are frequently reduplicated as the final channels are not always definitely selected. In order to show the different branches of the tree without extra illustrations, in this and the succeeding reconstructions, the pulmonary vein has been kept in the median line, and only the chief channels are shown in the case of reduplication, which is a frequent occurrence.

In a pig 15 mm. long, the pulmonary artery (Pl. II, Fig. 17) on the right side still has a more ventral and medial position than that on the left, a fixed relationship from embryos 12 mm. in length as the arteries both rise from a common trunk originating from the left pulmonary arch. Just below the point of origin of Lateral 1, the artery to that trunk is observed (Pl. II, Fig. 17), which passes up and divides with it into its ventrosuperior and dorsoinferior branches. The two pulmonary arteries bending dorsalwards pass back of the right and left bronchi, giving off the branches to the second lateral bronchi, which lie on their dorsal and superior surfaces. On the right side, the artery to the second ventral bronchus (Pl. II, Fig. 17) has increased in length with the growth of that branch, while arteries to the second dorsal bronchi (Pl. II, Fig. 18) are observed passing along their lateral walls. From this point, the pulmonary arteries continue on in the angle between the dorsal and lateral bronchi, giving off branches to the third and fourth lateral elements (Pl. II, Fig. 18) on each side which lie above and behind them. From the capillary plexus around the termination of the right and left stem bronchi, the beginnings of the pulmonary veins (Pl. II, Fig. 17) are seen as in the preceding stage. From the fourth lateral and third lateral branches on either side, veins are formed which lie below and in front of these bronchi and pass in front of the stem bronchi to empty into the pulmonary veins, which lie upon their median and ventral aspects. The vein from the second ventral bronchus (Pl. II, Fig. 17), as in the younger stage, is placed medially to it and empties into the right pulmonary at the base of the third lateral bronchus. The veins from the second laterals have increased considerably in length, and lie on the ventral aspect of these divisions, while the Vena pulmonalis, formed by the confluence of the two right and left veins, lies ventral to the trachea

just below the point of bifurcation. On the right side, the vein from Lateral 1 passes downwards and medianwards to empty into the Vena pulmonalis at a point just above the confluence of the two vessels which accompany the stem.

In a pig 18.5 mm. long, the relationships of the pulmonary arteries to the trachea (Pl. II, Fig. 19) remain the same. Just above the point of bifurcation, they pass gradually behind the main bronchi to take up their dorsolateral position. No marked changes are observed in the arteries to Lateral 1, save in an increase in length. The second lateral branches present no changes, except on the left side where a branch runs up on the dorsolateral aspect of the apical division of Lateral 2 (Pl. II, Fig. 19). The artery to Ventral 2 arising just beneath the Lateral 2 on the right side and passing around the stem and under the root of Lateral 2 to run along the outer aspect of the second ventral bronchus, now shows a secondary branch which follows the inferior division (Pl. II, Fig. 19) of Ventral 2. Small arteries are given off to right and left Dorsal 2 which run along their lateral superior aspect. On either side, branches to Lateral 3 (Pl. III, Fig. 20) run from a point just below the origin of the arteries of Dorsal 2. Beneath the third lateral bronchi, arteries arise which pass around the axial bronchus, and run lateralwards to Ventral 3. Below this level, branches are given off on both sides successively to Dorsal 3, Lateral 4, Ventral 4, Dorsal 4, and Lateral 5 (Pl. III, Fig. 20). The pulmonary veins (Pl. II, Fig. 19) lie medialwards and ventral to the main bronchi. Besides the branches from the lateral bronchi, which have been observed in the preceding stages, venules, lying on the medial surface of the dorsal bronchi, pass around the median aspect of the main bronchus and empty into the pulmonary veins. Similar veins from the ventral bronchi run along their median aspect, and empty into the Venæ pulmonales on both sides. Otherwise, there are no marked changes in the venous system at this stage save that the veins from the Lateral 2 and Lateral 1, on the right side now empty into the Vena pulmonalis by a common trunk. The second lateral vein on the left and with it a vein from the apical branch, which joins it about the root of Lateral 2 empties into the main pulmonary vein at a level somewhat higher up than the one which accompanies the left stem bronchus. The two veins from the stems join about the point of origin of the main bronchi and are continuous with the Vena pulmonalis above. From the infracardiac bronchus, a vein empties into the right stem vein just above the level of L. 3.

At this stage the main characteristics of the pulmonary vessels are established for life. The arterial branch to Lateral 1 runs upwards

from the right pulmonary artery along the ventral surface of the bronchus and then follows the main divisions of the bronchi. Both arteries pass down behind the stem, lying on their dorsolateral surface in the angle between the dorsal and lateral bronchi. From it, three series of vessels arise, namely, those to the lateral bronchi, which run on the dorsosuperior surfaces; those to the dorsal bronchi, which pass backwards from the stem artery on the laterosuperior aspect of the bronchus; and those to the ventral bronchi, which pass lateralwards around the stem bronchi to the lateral surfaces of the ventral group. Owing to the suppression of median bronchi on the tree of the 18.5 mm. embryo, the origin of the vessels to the median bronchi will be studied later in the corrosions of older embryos.

The veins have two chief branches accompanying the stem bronchi on their ventromedial surfaces. They receive as tributaries, veins from the lateral bronchi, which run along their ventroinferior surfaces and join the stem vein by passing above the corresponding ventral elements. Branches from the dorsal series of bronchi run along the medial surface of the bronchi across the median aspect of the stem to empty into the veins on either side. A series of tributaries are also derived from the ventral bronchi, which, after a short course on the medial aspect of these bronchi, terminate abruptly in the stem veins. The vein from L. 1 lies ventral to the corresponding artery and empties into the vein of Lateral 2 in the Vena pulmonalis. Thus we have the veins from the upper and middle lobe emptying together into the main Vena pulmonalis on the right, while the single vein from the upper left lobe joins the main trunk on the opposite side. Below, the veins accompanying the stem fuse just below the division of the trachea and empty at this point into the Vena pulmonalis. The moving of the veins towards the left, due up to this time to the asymmetry of the heart and the hyperdevelopment of Ventral 2, is now somewhat exaggerated by the development of the inferior vena cava on the right side of the infracardiac lobe, which also presses this structure to the left and, accordingly, must be looked upon as a factor in increasing the asymmetrical position of the pulmonary veins.

The next period of growth in the vascular system can be easily followed in specimens of the entire embryonic lung which, after fixation in some fluid like formalin or corrosive acetic to preserve the blood in the larger vessels, are subsequently cleared in oil of cloves or creosote. If the vessels are not too full both series are easily traced, but, in any case, the veins stand out distinctly. Owing to the complicated structure of the tree, however, the exact relationships of the arteries and veins to the bronchi are best seen in double corrosions in which, either the bronchi

and arteries or the bronchi and veins are injected, or else, in triple injections where all three systems are filled with different masses. Preparations with the artery and veins filled with one color and the bronchi another, are relatively easy to obtain, but the more instructive triple injections are extremely difficult to make. The changes gradually taking place with the growth of the tree, may be followed step by step in these cleared and corroded specimens, but they need not be described in detail until they are more exaggerated, as shown, for example, in triple corrosions of a pig 15 cm. long. Owing to my inability to find an artist who could draw these complicated structures, the reader may perhaps find it convenient to follow the following descriptions by means of the metal corrosions shown in Pl. IV, Figs. 23, 24. The common pulmonary artery now divides to the left of the trachea a short distance after its origin from the pulmonary arch. The branch to the tracheal bronchus is given off from the right pulmonary artery at the left margin of the trachea and, after crossing ventralwards to it, divides with Lateral 1 into a dorsoinferior and a ventrosuperior branch. The latter passes ventralwards to the tracheal bronchus, and, at its point of division, mounts up over the ventrosuperior branch and comes to occupy a position dorsal, slightly medial, to this bronchus. The dorsoinferior branch passes beneath, and runs dorsal to the dorsoinferior bronchus. The right pulmonary artery then passes downwards in front of the trachea, and turns back and out to occupy a dorsolateral position to the axial bronchus. Just above the second lateral bronchus, the branch to that division of the tree is given off, which courses a little above and behind the bronchus sending ramifications to accompany its side bronchi. The dorsoinferior branch crosses behind the main bronchus, and runs dorsal to the branch which it supplies, leaving that structure between it and the corresponding vein. In the remainder of its course, the second lateral branch lies dorsal to the bronchus with the bronchus between it and its accompanying vein. The branch to Ventral 2 originates just below Lateral 2 and, passing underneath its root, winds around the axial bronchus to gain the lower and lateral aspect of the Bronchus infracardiacus, which it accompanies in its ramification. The dorsal branch to Dorsal 2 runs on the lateral surface of the bronchus and is given off from the right pulmonary artery near the origin of the bronchus. The third lateral branch lies dorsalwards and sligthly superior to Lateral 3, and ramifies with its branches. The branch to the third ventral bronchus arises in a manner similar to that of the second, and winds underneath the third lateral bronchus around the stem to the lateral aspect of Ventral 3. The artery corresponding to Dorsal 3 has a similar distribution to the one above. The

fourth lateral lies above and behind the bronchus, while the fourth ventral passes in a similar manner to those supplying the same series of bronchi in the upper part of the tree. The fourth dorsal runs backwards just lateral to the bronchus, maintaining, in general, this position as it ramifies. In cases where there are median bronchi, as in this specimen, the artery passes medianwards around the dorsal surface of the stem and is placed dorsal to the bronchus during its ramification. The fifth lateral, ventral, and dorsal have corresponding positions to those of the higher orders, and occupy the same relative positions. On the left side the pulmonary artery passes down without crossing the left bronchus at all to take its dorsolateral position to the stem. Just above the point of origin of left Lateral 2, the corresponding artery arises, and after passing a short distance dorsosuperior to the bronchus, almost immediately divides, sending a branch to the apical bronchus which continues upwards, placed laterally and dorsally to it. The remainder of the arteries on the left side have the same course as the corresponding branches on the right. In this description, I have followed strictly the typical specimens, although it is well to bear in mind that here, as in other parts of the vascular system, frequent variations are encountered. The veins still unite to empty into the left auricle through a common Vena pulmonalis. Branches from Lateral 1 and 2 form a common, large venous trunk on the right side, emptying directly into the Vena pulmonalis, while the vein from the left Lateral 2 joins the latter at a corresponding level on the opposite side. Below, the veins accompany the stem bronchi and their tributaries form a common trunk at the level of Lateral 3, which, crossing the ventral part of the stem bronchus between Lateral 2 and 3, empties into the pulmonary vein from below. The further growth of Ventral 2 on the right has gradually pushed the veins from the lower portion of the bronchial tree much more to the left, so that the large common trunk from the portion of the tree below Lateral 2 lies directly over the left axial bronchus at a point where the second ventral bronchus on that side would originate if the latter were present. It is this fact, as we have pointed out above, which has such great significance in explaining the suppression of that branch. From the ventrosuperior branch on the tracheal bronchus, the vein lies ventral and medial to it, receiving tributary vessels placed somewhat below the side branches of this bronchus. The vein from the dorsoinferior branch of Lateral 1 is placed ventralwards to that branch, and passes upwards to join the main trunk at a higher level. The main vein from Lateral 1, then passes down ventral to the artery and bronchus to form a common trunk with that from Lateral 2 as we have described above. The latter

is placed above and ventralwards to the bronchus, receiving tributaries from its side branches. The main dorsoinferior branch of Lateral 2 lies ventralwards to its bronchus, while the corresponding artery is placed dorsalwards and above. This vein crosses behind Lateral 2 to join the main venous trunk, which accompanies Lateral 2 until, in common with the vein to the tracheal bronchus, it empties into the common pulmonary.

The veins from Lateral 3, 4, and 5 have shifted now so that they lie a short distance ventralwards from the corresponding bronchi. They pass medialwards under the ventral bronchi and empty into the right pulmonary stem vein; those from Ventral 3, 4, and 5 lie medial to the respective bronchi and run dorsalwards to the stem vein. Two veins now accompany Ventral 2, one above running medialwards and upwards and emptying into the large trunk formed by the fusion of the two stem veins, and another lying behind the branches of Ventral 2 which passes upwards and joins the common vein from the lower part of the tree on its right side at the point of junction of the veins from the right and left stems.

From Dorsal 2, 3, 4, 5, and 6 the veins, lying medial to their stems, run ventralwards past the stem bronchus to empty into the large stem veins opposite their corresponding branches. The veins from the medial branch lie ventralwards to them and pass lateralwards to the stem vein. The relationships of the veins on the left side of the tree below Lateral 2 are, with the exception of those from the Lobus infracardiacus, similarly arranged to those on the right.

Throughout the whole tree to this stage, we note with the single exception of Lateral 1 the constant relationship, which was indicated in the earlier embryos, of the regular alternation of artery, bronchus, and vein. In the earlier stages, the vessels were placed relatively close to the bronchi; but with the increasing age of the embryo, the position of the artery and vein has gradually shifted giving them a position at some distance from it. In some cases, this wandering may be so marked, especially below Lateral 2, that the main veins and their chief branches may occupy a position approximately midway between the adjacent bronchi. The arteries, however, always lie closer to the air passages.

In the first part of embryonic life, the left pulmonary arch with a portion of the right connects the right ventricle and the aorta, and the pulmonary arteries, after the manner described by Bremer, finally take origin from the left by a common stem arising from its under surface. The aortic arch lies above, and both arches are situated superior to the

point of origin of Lateral 1, the tracheal bronchus. This relationship and the subsequent behavior of the two arches as the heart moves down affords us, I believe, some suggestive hints to explain the suppression of Lateral 1 on the left side and its unusual low position in those animals in which it is present. Through all of the stages we have followed hitherto, both the aortic and pulmonary arches, and the origin of the pulmonary arteries lie well above the origin of Lateral 1. As shown by a corrosion of the bronchi, arteries and veins in an embryo 15 cm. long, the pulmonary arch is exactly opposite the site of origin of Lateral 1, while the aortic arch is still higher. At the age represented by a pig 20-21 cm. long, the heart and vessels have descended further caudalwards, leaving the pulmonary arch well below the root of Lateral 1 and the aortic arch exactly at its level. At the time of birth, both arches have descended still more and pass dorsalwards in the interval between the trachea, the stem bronchus, and the apical branch of Lateral 2 (cf. Pl. IV, Fig. 25). Now, had a symmetrical branch to Lateral 1 developed on the trachea, it is obvious that the descent of the great vessels and heart would have been prevented. Instead of reaching their final resting place just above the division of the trachea, they would have been left hanging above the level of Lateral 1. It is thus reasonable to suppose that the failure of this branch to form is due to a phylogenetic provision on the part of the tree to leave a passage for the descent of the heart and its great vessels.

A similar state of affairs is met with in the suppression of Ventral 2 on the left side. As the pulmonary vein forms approximately in the median line in the younger stages, the hyperdevelopment of right Ventral 2, the development of the inferior vena cava on the right side, and the shifting of the origin of the pulmonary vein from the site of its formation near the center of the undivided portion of the auricle to the left auricle, together with the increasing asymmetry of the heart, tends to carry the vein to the left. From its primitive approximate midline position in the earlier embryos, it is found with the increasing age of the embryo gradually passing to the left. In a pig 15 cm. long, we have the vein for the entire lower segment of the tree lying over the portion of the stem where left Ventral 2 should develop. Later still, in an embryo 20-21 cm. long, the descent of the heart has changed once more these relationships leaving this area of the stem bronchus covered by the root of the pulmonary vein as it empties into the left auricle. As in the case of Lateral 1, this suppression represents a provision on the part of the tree to leave a space for the pulmonary veins.

We are forced, however, to consider those animals in which these commonly suppressed elements are present. In these relationships we can see a reason why no Lateral 1 and Ventral 2 should form, but whether this stands absolutely in the relation of cause and effect, it is impossible from my material to say, as it is conceivably possible, although less probable for the condition to represent an adaptation on the part of the vessels to the use of unoccupied space. For either its absolute affirmation or disproof, therefore, a series of animals, in which these elements occur, must be examined from this standpoint during their developmental stages. This much may be said, however, in all of the lungs objectively pictured by Aeby, Huntington, and Narath where Lateral 1 is present on both sides, the one on the left is usually lower than the corresponding branch on the right. In the instances where they are on the same level, both are so low that they do not interfere with the descent of the heart and great vessels. Similarly, a bronchus that is not situated on the left stem in the segment between L. 2 and L. 3 cannot be considered as the homologue of V. 2, the Bronchus infracardiacus. All other cases are substitution branches of the lateral bronchi or the stem. In the lungs which have been well pictured in the literature, where a real Ventral 2 occurs on the left stem, they are usually small and poorly developed and would not materially influence the migration of the Vena pulmonalis. It is also possible in these cases, as the veins are never drawn, that the latter have different relationships from those

Influence of the Vessels upon the Architecture of the Bronchial Tree.— After following the development of the vascular system, we may consider now the possibility of the influence of the vessels upon the architecture of the bronchial tree. Concerning the general asymmetry of the lungs, many of the older investigators have looked upon the heart or the great vessels as being responsible for this irregularity. Thus Bichat, 29, and Rüdinger, 73, thought the left bronchus owed its greater length to the asymmetry of the heart, while Meyer, 61, looked upon the aortic arch as the factor which drew it out to greater length. In reviewing these statements, Aeby felt these authors passed over the most weighty relationship in overlooking the crossing of the bronchi by the arteries at a particular point on the stem to run down on its dorsal surface. This crossing enables the artery in the "hyparterial" to divide the side bronchi into a dorsal and ventral series, while the "eparterial" bronchi, situated above this separating influence of the artery, have their dorsal and ventral branches arising from a common stem. In quoting Kölliker's observations on a 35-day human embryo, Aeby calls attention

to the origin of the pulmonary arteries in the embryo above the lungs, and states: "Ein später eparterieller Bronchus muss somit so lange hinter ihr liegen, als nicht in Folge des höhern Aufsteigens des Organs eine bogenförmige Ablenkung derselben über den ersten Ventralbronchus hinweg nach vorn hin stattgefunden." While Aeby looked upon the lungs instead of the heart as the movable factor in establishing the adult relationships of the arteries to the tree, he recognized notwithstanding this misinterpretation, the necessity of the embryological topography of the "eparterial" or first lateral bronchi to produce the conditions which we find in later stages. It is clear from the above account of the development of the pulmonary arteries that these delicate vessels which regularly follow the growth of the bronchi and do not, in fact, appear in any part of the lung until after the respective branches which they supply are present, have no formative influence on either the structure or relationships of the bronchi, but are simply passive followers of their development produced by histomechanical principles from the capillary plexus which surrounds them. Finally, a crossing of the stem bronchus by the artery does not occur until after birth when all of the bronchi are laid down, and even then, in the strict sense of Aeby, does not exist as Zumstein and Narath have already shown. It is thus most difficult to determine just what led Aeby to lay such stress upon the adult relationship of the artery to the stem when he obviously, as the above quotation shows, clearly recognized that it was not associated with the earlier formation of bronchi, but was due, as he supposed, to the later ascent of the lungs. Furthermore, the pulmonary artery is not responsible for the dorsal and ventral divisions of the stem bronchi as we have ventral and medial elements also arising from the stem away from any possible influence of the artery.

Müller, 98, brings forward an interesting suggestion with reference to the effect of the pulmonary arteries on the tree dependent upon the descent of the heart in mammals which have had the form of their chest wall altered by their life in water. The pulmonary arteries, according to Müller, following the descent of the heart tend to drag the "Ventral bronchi" caudalwards, leaving the dorsal bronchi free and uninfluenced by the arteries to wander up on the stem bronchus or trachea to form the so-called "eparterial" bronchi. This ingenious suggestion is not borne out, however, by the facts of embryology, for as we have seen, all the bronchi are well formed before the heart in its descent reaches a level where the pulmonary arteries could exert such a traction upon the lateral bronchi.

Huntington, 98, says: "If we seek for an explanation of the cause which leads to the migratory changes of the cephalic bronchus (Lateral 1), I admit that we enter the realm of pure hypothesis. At the same time, the very general development throughout the mammalia of this type, with the resulting greater respiratory area of the right lung, may, I think, not improperly be referred to the development of the mammalian form of the systemic and pulmonary arteries. On the left side, the greater quantity of blood thrown from the right ventricle into the left pulmonary artery passes through the Botallian duct directly into the aorta, only a small portion traversing the left pulmonary circulation. On the right side, however, with the early obliteration of the dorsal segment of the fifth arch, all the blood entering the right pulmonary artery is forced to traverse the entire pulmonary circulation returning to the left auricle by the pulmonary veins." This explanation, according to Bremer's description of the development of the pulmonary arteries, could not account for the increased size of the right lung, especially in the pig where all of the blood to the lungs is forced to pass through the left pulmonary artery after the establishment of the transverse anastomoses and the subsequent degeneration of the proximal portion of the right pulmonary artery.

We may say then in conclusion, that there is one simple possible explanation for the general asymmetry of the mammalian lung which lies in the asymmetry of the anlage. Owing to the fact, however, that the pulmonary anlage in lower animals is frequently symmetrical, it seems more probable to look upon this characteristic as an adaptation on the part of the pulmonary apparatus to its environment which may reach such extremes as we find in the lung of the snake. It is more probable then, that, with the necessity of an increased respiratory surface as we ascend the animal scale, the asymmetrical heart and the development of its adult form gives us adequate ground for a normal asymmetry of the respiratory apparatus, especially as the heart and liver, forming the principal environment of the lungs, have phylogenetic precedence and are of more physiological importance during intrauterine life. In its final form, this asymmetry consists, in the vast majority of lungs, in a suppression of left Lateral 1 to leave space for the descent of the aorta and pulmonary arch with the heart and a suppression of left Ventral 2 to provide room for the pulmonary veins from the lower lobes. In animals, however, where these branches are formed they are so placed that they do not interfere with either of these features of the development of the vascular system.

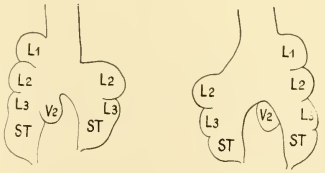
Lobe Formation in the Lungs.

The relation of the mesoderm to the primitive tree has been described in connection with the appearance of the bronchi, largely because it arises from the general mesoblast of the head gut and takes part in the separation of the pulmonary anlage from the œsophagus. The meso-

Text Fig. 15.

Text Fig. 15. Outline drawing of the lungs of an embryo pig 10 mm. long. Ventral view. (Figs. 15-19-24, inclusive, drawn with a camera lucida from cleared preparations.) *L.* 1, *L.* 2 = Swellings, limited by shallow grooves, over Lateral 1 and Lateral 2. *ST* = Mesoderm over the caudal portion of the stem bronchi. Also *L.* 1 = Lobus superior. *L.* 2 = Lobus medius (right) and Lobus superior (left). *ST* = Lobus inferior.

derm, it will be remembered, shows the influence of the first irregularity of the early branches of the tree and forms two indefinite unequal rounded projections into the primitive cœlom on either side. These



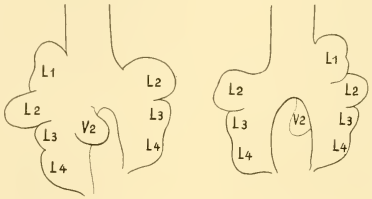
Text Fig. 16.

Text Fig. 16. Outline drawing of the lungs of an embryo 12.5 mm. long. A. Ventral view. B. Dorsal view. *L.* 1, *L.* 2, *L.* 3, *V.* 2, and *ST* = Swellings over the several bronchi and the stem designated by these abbreviations. At this stage the anlagen of the lobes are complete. *L.* 1 = Lobus superior, *L.* 2 = Lobus medius (right), Lobus superior (left). *V.* 2 = Lobus infracardiacus. *L.* 3 and *ST* = Lobus inferior.

are the anlagen of the two lung wings. On both sides the Recessus pleuroperitonealis projects upwards and somewhat medialwards to the bronchi; the left, however, is very poorly developed. Ventralwards the mesoderm continues forwards into the Mesocardium posterior.

At 10 mm. the two simple lungs are quite asymmetrical (Fig. 15). Increasing in size with the growth of the bronchi, they also follow their asymmetrical development. The fain swellings observed in the preceding stage have become so exaggerated that we have on the surfac of the lung marked rounded elevations indicating the presence of Lateral 1 (Fig. 15, L. 1) on the right side, and Lateral 2 on both sides (Fig. 15, L. 2). These projections are limited by shallow groves. From above downwards, the trachea and hence the mesoderm extends ventralwards until the point of bifurcation is reached, when, following the course of the stem bronchi, it passes dorsalwards on either side of the œsophagus.

At 12.5 mm. (Fig. 16) these characteristics are exaggerated. On the right side, high up, we have the projections over the bronchi, which have been found before this stage. They have increased in size with the



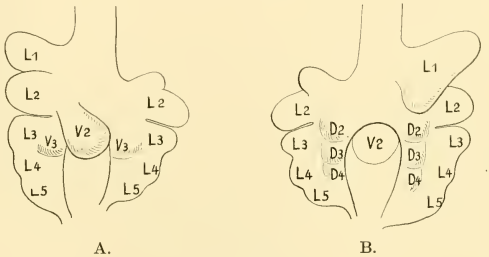
Text Fig. 17.

Text Fig. 17. Outline drawing of the lungs of an embryo pig 13.5 mm. long. A. Ventral view. B. Dorsal view. The letters represent the mesodermic swellings over the bronchi designated by the abbreviations. Designations the same as in Fig. 16, except that L. 3 and all swellings below that order unite in the pig to form the Lobus inferior.

growth of their respective elements; also there is now a well-marked projection over the newly-formed V. 2 (Fig. 16, V. 2) and a less apparent swelling, the bud representing Lateral 3 on each side (Fig. 16, L. 3). The furrows have deepened, and the lower part of the wings below Ventral 2 now embraced by the Wolffian body and chest wall dorsally, the heart, liver, and diaphragm ventrally, and the mesoderm of the œsophagus medially, have already in cross-sections an irregular prismatic form. At this stage we may say, the anlagen of the lobes are complete. From each of these main projections, a lobe is produced and the shallow grooves deepen with the further growth of the lungs to form the interlobar fissures. That is to say, on the right side the swellings over Lateral 1, Lateral 2, Ventral 2, and the stem produce respectively

the Lobus superior, Lobus medius, Lobus infracardiacus, and Lobus inferior, while Lateral 2 and the stem bronchus produce the Lobus superior and Lobus inferior on the left. At 10 mm. the swelling over L. 1 is practically in the same lateral plane as L. 2, while at 12.5 mm. it is crowded slightly dorsalwards by the further growth of the latter.

In a pig 13.5 mm. long (Fig. 17), the characteristics of the lobe formation are intensified. On the right side, the upper lobe containing Lateral 1 is pushed still more dorsalwards, while the middle lobe containing Lateral 2 is, at the same time, forced slightly ventralwards by the antagonism in the growth of their two main bronchi. The Lobus infracardiacus, containing Ventral 2, extends downwards and medialwards, while the lower lobe extends more caudalwards and is now, through its whole extent, distinctly prismatic in cross-section. On the



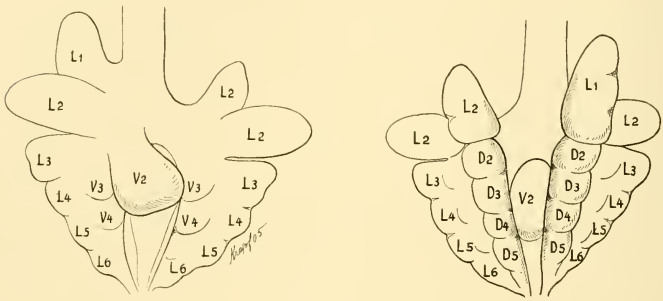
TEXT FIG. 18.

TEXT FIG. 18. Outline drawing of the lungs of an embryo pig 14.5 mm. long. A. Ventral view. B. Dorsal view. Designation of lobes as in Fig. 16.

left side, the Lobus superior, owing to its more unobstructed environment, extends somewhat higher than its homologue, the Lobus medius, on the right side. The Lobus inferior is not quite so large or well developed as the corresponding right lobe. The primary fissures between the several lobes have deepened and now extend well into the substance of the lung. With the division of Lateral 1 and Lateral 2 on each side, the secondary branches also raise secondary projections on these surfaces of the lobes between which are slight secondary furrows. Similarly the Lobus inferior on each side shows slight swellings limited by shallow grooves over L. 3 and L. 4. In the pig, these swellings and grooves, however, under ordinary circumstances, never lead to a separation of the lung substance into extra lobes.

Fig. 18 shows the lungs of an embryo 14.5 mm. long. The Lobus

superior on the right side (Fig. 18, L. 1) is now pushed dorsalwards by the presence of the heart and the Lobus medius (Fig. 18, L. 2), so that its caudal portion now lies above the series of swellings over the dorsal bronchi (Fig. 18 *B*, D. 2). On the left side, the Lobus superior now shows a dorsoapical swelling over the apical branch of L. 2 (Fig. 18, L. 2), which indicates the beginning of the portion of the left upper lobe, which substitutes for the Lobus superior on the right side. The fissure between L. 2 and L. 3 on each side deepens, while the Lobus inferior on both sides shows a series of projections over the several branches of the stem. On the ventral surface, V. 3 is indicated; on the lateral border,



Text Fig. 19.

Text Fig. 19. Outline drawing of the lungs of an embryo pig 18.5 mm. long. A. Ventral view. B. Dorsal view. The abbreviations on the swellings represent the order of the bronchi beneath. Designations as in Fig. 16.

L. 3, L. 4, and L. 5; while, on the dorsal border, swellings for D. 2, D. 3, and D. 4 are present.

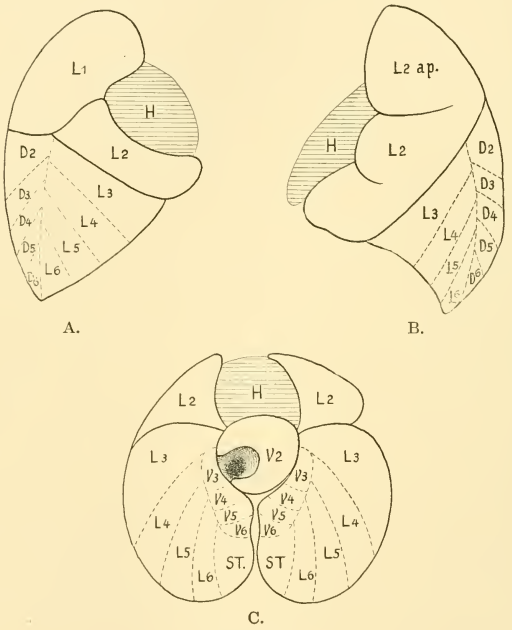
In a pig 18.5 mm. long (Fig. 19), the right Lobus superior containing Lateral 1, projects upward some distance beyond the tip of the upper lobe on the left side. The fissure separating it from the Lobus medius has deepened. Its lower portion now passes behind the medial lobe, although the two are united at their roots, that is to say, the ventromedial aspect. The Lobus infracardiacus projects ventralwards and medialwards until it extends over the median line above the œsophagus. The lower lobe on the right side shows projections along the lateral border for L. 3, L. 4, and L. 5, and, on the dorsal border, for D. 2, D. 3, and D. 4. The ventral surface, likewise, has very slight swellings for

V. 3 and V. 4. The latter, however, are very faint and are separated from the rest of the lobes by very shallow grooves. On the left side, the Lobus superior (Fig. 19, L. 2) is separated from the lower lobe by a deep cleft, while the development of the apical branch of L. 2 has pushed up with it a segment of this lobe which also grows backward until it lies above the series of dorsal swellings (Fig. 19 *B*) and bears a marked resemblance to the Lobus superior on the opposite side. Excepting for the Lobus infracardiacus, the lower lobe has characteristics practically homologous to the corresponding lobe of the right side. The dorsal flexion of both lower lobes still persists and the lateral tips or margins of the median lobes now begin to show, at their lateral extremities, a slight bending ventralwards as they begin to fold around the heart.

As the lung continues to grow, with the successive appearance of new branches, new elevations are formed on the surface of the primitive lobes until finally, as Narath describes, they have an appearance like the surface of a mulberry. The primitive lobes, however, keep their independent character and alter in form by two chief factors, namely, the intrinsic growth of the lung itself, and the change in its environment formed by the chest wall, heart, liver, and diaphragm. Narath has given as the cause of the lobe formation, the extremely rapid growth of the first branches of the tree, while the later branches of slower growth fail to form furrows in the mesoderm deep enough to subdivide the lung further. With this view, I am in complete accord, but it ought, it appears to me, to be extended to include the character of the mesoderm. In the early stages, this is in extremely plastic form, which easily moulds itself to the pressure of the growing bronchi beneath. Up to 10 mm. there is scarcely any differentiation in the mesoderm into distinctly fibrillar and cellular portions, while at 12 mm. this change is inaugurated and fibrils appear particularly in the region of the root of the primitive lung. At 20 mm. the whole mesodermic portion is composed of young connective tissue with well-marked fibrils. As the mesoderm differentiates, therefore, it becomes firmer and is less easily influenced by the growth of the young bronchi.

Fig. 20 is an outline drawing of the lateral and diaphragmatic aspects of the lungs of an embryo 19 cm. long. At this time, all of the important adult topographical features of the lungs are present. A. shows well how the right Lobus superior has grown down and back into the dorsal area, moulding itself even more than in an embryo 18.5 mm. long (Fig. 19) to that portion of the thoracic cavity and extending now up over the

base of the heart beyond the midline making the sum of lung tissue in L. 1 and L. 2 considerably greater than that in L. 2 on the opposite side. Owing to this growth, the Lobus medius is pressed ventralwards, its dorsal segment lying in the angle between the Lobus superior and the Lobus inferior. It may be interesting to note, that the portion of the



Text Fig. 20.

Text Fig. 20. Outline drawings of the lungs of a pig 19 cm. long. A. Right side. B. Left side. C. Diaphragmatic surface. At this stage, the surface of the lungs is smooth. The topography of the bronchi beneath, taken from corrosion specimens of the same age, is indicated by dotted lines and letters.

lobe which lies in this angle, is supplied by the large dorsoinferior bronchus. It is, therefore, ontogenetically equivalent (*vide* Pl. II, Figs. 15, 16) to the apical segment of the Lobus superior on the other side. Nothing could indicate clearer the adaptation of the growing

bronchi to their environment, or the possible influence of environment upon the branches of the tree. The tips of the Lobus medius have grown around the heart until they have almost met in the midline. On the undersurface, the unpaired Lobus infracardiacus (Fig. 20, V. 2) is clearly seen particularly in its relationship to the Vena cava inferior.

With the increase in size between this and the last stage, the swellings over the various bronchi have disappeared and the surface of the lobes become smooth. The topography of the Lobus inferior on both diaphragmatic and lateral surfaces is indicated on the surface of the lungs by dotted lines. By a comparison with Fig. 19, the origin of these topographical relations are clear.

With the further development of the pig's lung which has been described by Narath, I cannot agree. In the account of the form relationships, his work is accurate, but in the interpretation of the relative significance of the different parts of the lung and the equivalent values of the lobes on each side, our results differ chiefly with our derivation of the principal bronchi. That is to say, according to his view the Lobus superior and the Lobus medius on the right side are equivalent to the Lobus superior on the left. They are almost or completely separated through an accessory fissure, making the Lobus superior correspond to the dorsal or apical area in his preparations and equivalent to the cephalic or apical projection of the Lobus superior of the left lung. The latter, as we have seen, is only a secondary substitution product of a branch of left L. 2, ontogenetically equivalent to the region of the Lobus medius on the right side which is supplied by the large dorsoinferior bronchus. On the other hand, the right Lobus superior, supplied by L. 1, is totally unrepresented in the left lung. This unpaired lobe, therefore, and also the cephalic portion of the upper lobe on the left, properly belong not to the dorsal area, as Narath suggests, but to our lateral and his ventral region. The fissure between Lobus superior and Lobus medius on the right would be primary and not accessory in the sense of Narath.

In recapitulating the development of the lobes, we may say, then, that the mesodermic portion of the lungs, derived from the general mesoderm about the head gut, is pushed out by the growing bronchi to form irregular asymmetrical swellings in the cœlom. These are the anlagen of the primitive wings of the lungs. With the appearance of L. 1 on the right side of the trachea, and L. 2 on each stem bronchus, primary swellings are formed in the two wings over these bronchi, giving rise to the simplest form of the Lobus superior, Lobus medius on the right side, and the Lobus superior on the left. The remainder of the mesoderm

about the stem bronchi form the anlage of the Lobus inferior on each side. With the appearance of V. 2, the Bronchus infracardiacus, on the right, a swelling forms over it yielding the anlage of the Lobus infracardiacus. These swellings are at first surrounded by shallow grooves, which, with the rapid growth of the bronchi beneath, develop into the fissures separating the various lobes. With the further growth of these chief bronchi and the appearance of the series of bronchi on the stem, a series of swellings and fissures are formed over and between them. These are equivalent, in all senses except in age and size, to the earlier fissures and swellings, but, under ordinary circumstances, never deepen into distinct lobes. This is partly due to the more rapid growth of the first bronchi, to the gradual increasing density of the mesoderm, and, lastly, to the environment of the several lobes of the lung. That is to say, the Lobus superior with L. 1 has the territory between the chest wall and the upper part of the heart on the right side. The right Lobus medius and the left Lobus superior, with L. 2, have the large space between the chest wall and the angle formed between the heart and liver on each side. It is important, however, to note on the left side, owing to the absence of L. 1, the Lobus superior sends up the apical segment of the lung containing the left Bronchus ascendens. The Lobus infracardiacus, with V. 2, grows out into the space left between the heart and liver and the two lower lobes, while the Lobus inferior on each side lying in the more or less triangular space between the chest wall and liver and diaphragm becomes prismatic in cross-section and grows caudalwards and lateralwards to fill up the rest of the pleural cavity.

In the pig, then, we have a series of primary projections limited by a series of fissures some of which give rise to the permanent pulmonary lobes. Those projections and fissures which take part in the lobe formation in the pig, it is well to observe, are the first to form, but in other animals these same conditions do not appear to obtain. In Hystrix cristata, for example, not only the primary fissures between practically all of the principal bronchi may give rise to a series of lobes, but these may even be subdivided by the secondary fissures formed by the secondary branches of these elements, while in other animals, as for example man, the deepening of the fissures about V. 2 usually do not produce a separate lobe, leaving this region of the lung included in the right Lobus inferior. Between these forms we have extensive individual and general variation.

The drawings in Fig. 20 may be used conveniently to explain the lobe production in all mammals; *A* represents the conditions in animals where L. 1 is present on one side or both; *B*, the conditions where L. 1

is absent on one side or both; *C* represents lungs where a Lobus infracardiacus is present, and by eliminating this lobe and altering the topography of the ventral bronchi, it may be used for lungs where V. 2 is either absent or included in the Lobus inferior. For example, *B* represents the conditions found in Hystrix cristata in both lungs where not only all of the primary bronchi in that animal have produced lobes, but some of them are still further partially subdivided. There is also a type of lung represented by Phoca vitulina where L. 1 is present on both sides, but L. 2 in this species is thrown into the Lobus inferior. For this state of affairs *A* would suffice if the permanent fissure between L. 2 and L. 3 were replaced by a dotted line. The suppression of the lobes indicated in Phoca vitulina may involve all fissures giving us a lobeless lung like those of Delphinus delphys and Pithecus satyrus.

It is, of course, clear from the above description how we regard the equivalent values of the lobes on the two sides, but they may be simply stated in two simple formulæ of equivalence which will fit the lungs of most animals depending upon the presence of L. 1 and V. 2 on one or both sides. Type 1 includes the great majority of mammalian lungs.

Type 1.

L. 1 present only on the right side.

Right Side.	*Left Side.*
Lobus superior = *O*.	
Lobus medius = Lobus superior.	
Lobus inferior = Lobus inferior.	
or	
Lobus inferior + V. 2 = Lobus inferior + V. 2 or *O*.	

Type 2.

L. 1 present or absent on both sides.

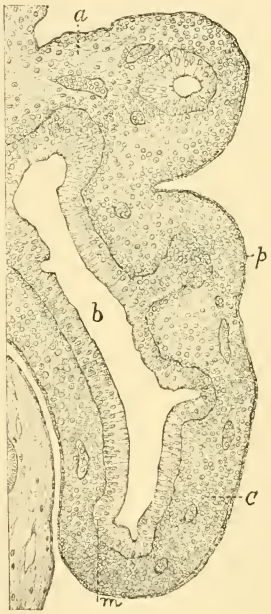
Right Side.	*Left Side.*
Lobus superior = Lobus superior.	
Lobus medius = Lobus medius.	
Lobus inferior = Lobus inferior.	
or	
Lobus superior = Lobus superior.	
Lobus inferior = Lobus inferior.	

While lobe production in the lungs is obviously dependent on the growth of the bronchi in the majority of instances, the number of lobes is apparently without definite morphological significance. It may vary in animals from multilobed lungs like those of Hystrix to lobeless lungs like those of Pithecus satyrus. The common relationships, however, are expressed in the types given above.

The Organogenesis of the Lungs.

In turning to the organogenesis of the lungs from the period of the formation of the Anlage until the adult stage is reached, the first interest settles in the chief cells of the bronchi and the pulmonary connective tissue. Both of these structures have been followed up to the age represented by a pig 10 mm. long, in the chapter on the development of the

bronchi. From this time, it is more convenient to consider these stages by themselves. In the description of the differentiation of the framework, I have taken as a basis the work of Mall, 02, who has described in the pig the origin of the connective tissues from a common mesodermic syncytium. By a differentiation of this syncytium into an endoplasmic and exoplasmic portion, the connective tissues are produced. The former



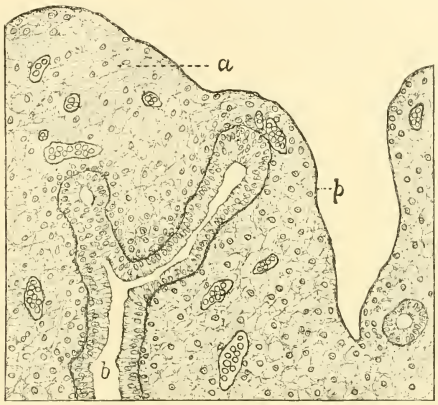
Text Fig. 21.

Text Fig. 21. Longitudinal section of the left lung of an embryo pig 13 mm. long. Fixed in Zenker's fluid and stained by Mallory's Fuchsin-Anilin blue method. × 70. *b* = Stem bronchus. *p* = pleura. *a* = Young connective tissue. *c* = syncytium. *m* = evagination forming medial bronchus.

remains as the protoplasm about the connective tissue cells, the latter forms the various fibrils. The author, 03, has traced the development of the framework of the submaxillary gland in the pig, where, in the earlier stages, the process of differentiation is the same as in the lungs. By

way of review, suffice it to say that the syncytium forming the primitive framework of the lungs differentiates slowly until 10 mm. is reached when, in the neighborhood of the root of the lung and the Mesocardium posterior, the fibrils begin to appear and the cells become more isolated from each other. About the young bronchi, however, they are still in close apposition during the formation of the reticulated membrane about the tubes, which, in Mallory preparations, may be seen as a dark blue line.

At 13 mm. (Fig. 21) these conditions are well shown. The stem bronchus (Fig. 21 *b*) and its chief lateral branches is seen in longitudinal section lined, by an epithelium consisting of a row of inner

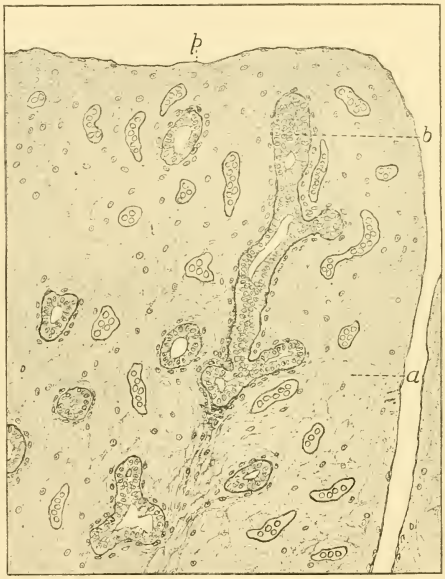


Text Fig. 22.

Text Fig. 22. Section of the lung of an embryo pig 3 cm. long. Same preparation as used with tissue shown in Fig. 21. × 70. *p* = pleura. *a* = connective tissue. *b* = bronchus.

columnar cells with smaller polygonal cells beneath them. The epithelial tube is surrounded by a simple reticulated membrane which is in process of formation. Above, at the root of the lung (Fig. 21 *a*), the transformation of the exoplasm into young connective-tissue fibrils has taken place, while in the lower portions of the Lobus inferior (Fig. 21 *c*), the framework consists of a mass of anastomosing syncytial cells without any particular differentiation. About the basement membrane, the cells are thickly packed and under the primitive pleura (Fig. 21 *p*) the epithelium of which has begun to flatten, we have a distinct blue line indicating the formation of a membrana propria.

In a pig 30 mm. long (Fig. 22), the framework of the entire lung shows a differentiation into primitive fibrils. The young fibrils are more distinct and less granular, while the spaces between are larger than in the preceding stage. With the differentiation, the relative quantity of endoplasm has diminished in the loose part of the syncytium, leaving in some places isolated connective-tissue cells (Fig. 22 *c*), or in



Text Fig. 23.

Text Fig. 23. Section of the lung of an embryo pig 5 cm. long. Same preparation as used with tissue shown in Fig. 21. × 70. *p* = pleura. *a* = connective tissue. *b* = bronchus.

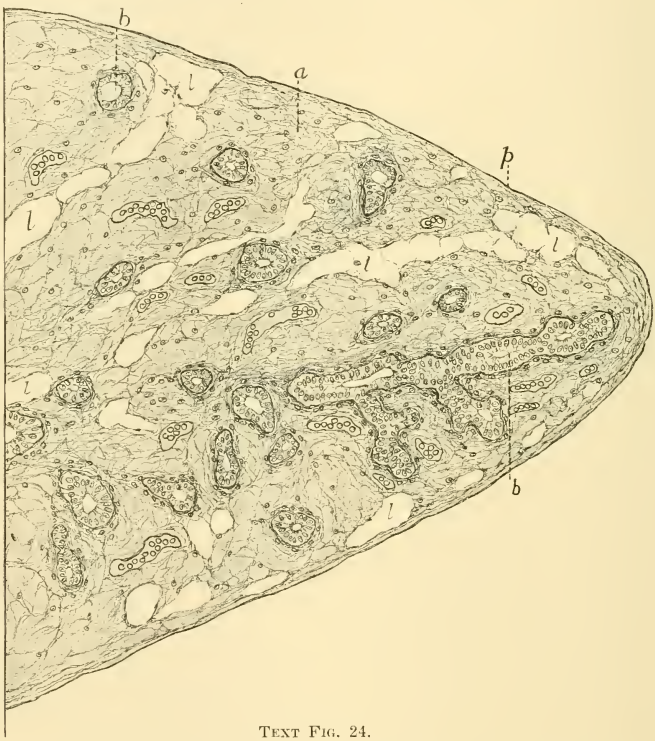
others they are multipolar in appearance with branching and sometimes anastomosing processes. Immediately about the trachea and large bronchi, the cells are closely packed together preparatory to the production of the various coats of these structures. The basement membrane is distinctly fibrillated as is seen at points where the plane of section is tangential to the bronchi. About the larger bronchial elements a

group of elongated fusiform cells having a distinctly circular arrangement may be noted, representing the earlier stages of the production of the muscular coat.

The epithelium in all the large and in the majority of small bronchi still consists of two layers of cells, the inner columnar, the outer polygonal in form. But in the youngest branches of the oldest bronchi, namely Lateral 1 or 2, there is now a reduction to a single layer of columnar cells (Fig. 22 *b*). Cilia are as yet invisible in these specimens, but the cuticula at the inner margin of the cells is already differentiated. At the root of the lung, a few dilated lymphatics may be noted near the bronchi and pulmonary vessels; they have not, however, grown beyond this point into the substance of the lung wings.

Embryo 5 mm. long (Fig. 23). The general framework (Fig. 23 *a*) of the lung at this period has undergone a further differentiation over the preceding stages, consisting in an increasing density and complexity of the young fibrils, which now possess a more distinctly fibrillar appearance, while the quantity of endoplasm about the connective-tissue cells has slightly diminished, except in the immediate neighborhood of the larger bronchi. The pleural epithelium (Fig. 23 *p*) is much more flattened and the nuclei of the individual cells consequently further apart. As shown by points where the plane of section falls tangential to its surface, the basement membrane beneath this epithelium is distinctly reticulated. About the larger bronchi, there is a distinct circumferential arrangement of the exoplasmic fibrils in which are imbedded a great many cells. The basement membrane is slightly thickened and just beneath the latter there is now a well-marked layer of fusiform cells with elongated nuclei running circularly about the bronchial tube. External to this stratum, is a looser circular arrangement of the exoplasmic fibrils as well as the cells embedded in it. When the bronchi are cut longitudinally, these circumferential lamellæ of cells and exoplasm run parallel to the long axis of the tube. The epithelium, as in the preceding stages, shows a distinct division into two or three layers, with the nuclei situated approximately in the middle of the cell. The thickening on the edge of the cell lining the lumen is apparent, although cilia are as yet unformed. As the branches of the tree are followed towards the periphery, the layers of circularly directed syncytial cells disappear and we have simply the primitive basement membrane with the connective-tissue cells immediately about it. In the most terminal parts of the air passages, the double layer of epithelium has been replaced by a single layer of lower columnar epithelium (Fig. 23 *b*). All of the bronchi

from the first to last possess marked lumina. From the root of the lung, the lymphatics have now grown some distance into its substance. They have thin walls composed of young fibrils lined by endothelium with occasional valves. They are confined, however, to the immediate neighborhood of the main bronchi and their chief subdivisions.



Text Fig. 24.

Text Fig. 24. Section of the lung of an embryo pig 7 cm. long. Same preparations as used with the tissue shown in Fig. 21. × 70. p = pleura. b = bronchus. a = connective tissue. l = lymphatics. This stage shows the beginning of the lobulation.

Pig 7 cm. long (Fig. 24). A number of interesting changes have taken place in the evolution of the lungs since the last stage other than in a further differentiation of the framework, which at this time is con-

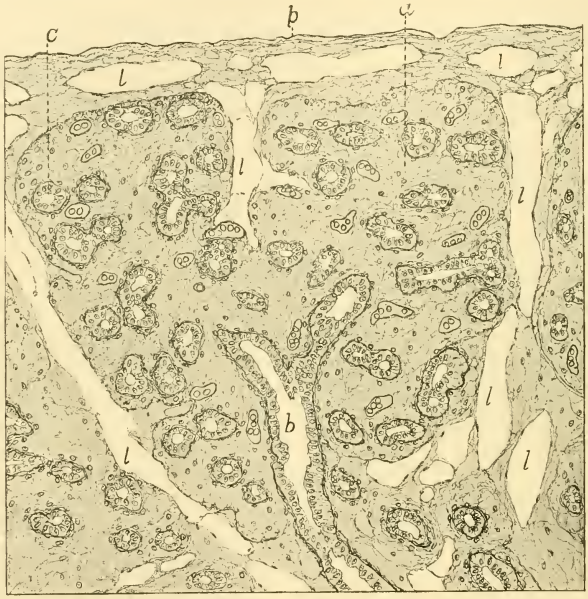
siderably denser. The circularly arranged fusiform cells noted in the earlier stages about the main bronchi are collected into bundles to form the muscular layer outside of the mucosa, while still external are stages in which the chondrification of the syncytium is progressing as the latter passes over into the precartilage stage at the periphery, and into young cartilage in the center to form the simple chondral rings of the trachea and larger bronchi. The epithelium of the latter is sometimes thrown into folds, is cylindrical, and composed of a double layer of cells. As one follows the branching to the end buds, it first becomes single layered and then of a low columnar type (Fig. 24 *b*). Chondral rings and bronchial cartilages are present only around the trachea and the upper part of the stem bronchi; the muscular coat, as one passes peripheralwards, thins out until it first consists only of a single layer of cells, and finally at the smaller branches and end buds is replaced by the young connective tissue, which, in the latter region, is engaged in the formation of the reticulated membranes.

The most interesting change, however, lies in the further growth of the lymphatics, which, in the earlier stages, are found in the root of the lung in the neighborhood of the pulmonary vessels and large bronchi. As they grow in, they accompany these structures for a distance, then, approaching the end branches, they leave them and run in a plexiform manner midway between the bronchial tubes (Fig. 24 *l*) until they reach the pleura (Fig. 24 *p*). This gives the lung now an indefinitely lobulated appearance, in which the periphery of the simple lobule is indicated by the lymph vessels and the center by the bronchi. The lymphatics are lined by flattened endothelium, their walls are formed by the young connective-tissue fibrils, and, here and there, valves are beautifully shown, which, in general, point away from the pleura. The pleural epithelium (Fig. 24 *p*) is much flattened and now rests upon a thickened layer of young connective-tissue fibrils.

Pig 13 cm. long (Fig. 25). At this stage, we have the whole lung subdivided into a series of connective-tissue lobules with essentially the same characteristics as those shown in the preceding stage, namely, a peripheral plexus of lymph vessels with the bronchus in the center. The growth is centrifugal in so far as the bronchi are concerned and, in this sense, the lung at this stage may be compared in some respects with the younger stages of the salivary glands for example, where similar lobules without peripheral lymphatics are also formed from a centrifugal growth of the ducts. The framework at this stage (Fig. 25 *a*) is considerably thicker than in the preceding embryo, the fibers denser and, at

the same time, there are more connective-tissue cells. Under the pleura (Fig. 25 *p*) and in the interlobular spaces, the fibrils are gathered into slight trabeculæ, which limit small spaces in the connective-tissue network.

The larger bronchi show an increase in the characteristics indicated in the last stage. The epithelium is thrown out into longitudinal folds,



Text Fig. 25.

Text Fig. 25. Section of the lobule of the lung of a pig 13 cm. long Same preparation as used with the tissue shown in Fig. 21. × 70. *p* = pleura. *a* = connective tissue. *b* = bronchus. *c* = end bud. *l* = lymphatics.

which are accompanied by folds of the basement membrane and submucosa. This is composed of trabeculæ formed from the young connective-tissue fibrils. In the young submucosa, the simple muscle bundles lie, and still external to the muscularis the cartilagenous rings are in process of formation. Proceeding peripherally, the bronchi grow essentially younger and the epithelium is first reduced to a single columnar

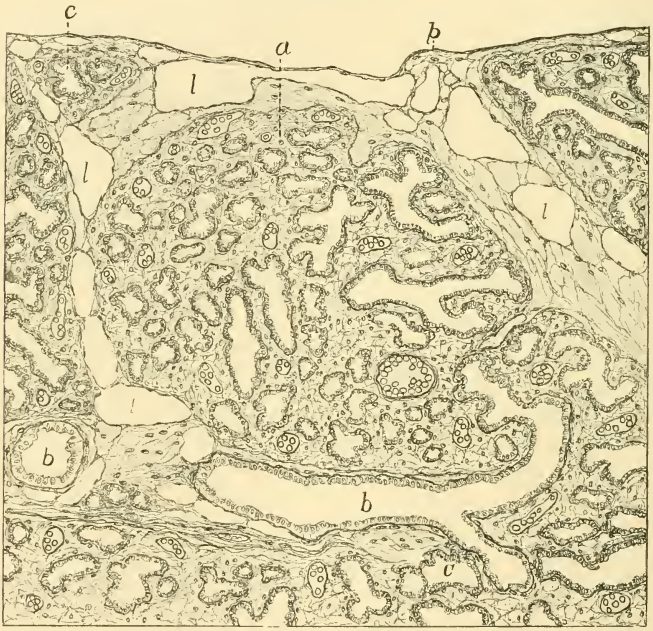
layer, which then becomes lower until, in the lobules, it forms a lower columnar epithelium. Still further out in the growing terminal buds (Fig. 25 *c*), it now has a distinct cubical form. About these, the membrana propria is formed from the connective tissue of the lobule.

The lymphatics (Fig. 25 *l*), forming a plexus around the bronchial veins and arteries at the root of the lung, accompany them towards the periphery, giving off branches to the interlobular spaces en route. Their walls, owing to the increasing differentiation of the framework, are thicker. On reaching the periphery of the lung, they leave these structures and pass out as in the preceding stages to the pleura. They have a plexiform arrangement and may (Fig. 25) be traced at times into the substance of the lobules. This course may also be observed in the deeper lobules of the lung as well as those on the surface under the pleura.

In the period of embryonic life between pigs 13 and 19.5 cm. in length there are no marked changes of the relationships we have thus far described. In the larger bronchi, a gradual development has occurred. The epithelium now possesses well-marked cilia springing from the cuticular border of the inner layer of epithelium, between the elements of which, goblet cells appear here and there, partly filled with mucous. These are clearly seen first in the stem bronchi of pigs between 15 and 17 cm. long. The folds, which have already been described running longitudinally with the bronchus, now look in cross-sections like regular papillæ with a core of submucosa. That they are regular structures of the bronchi and not shrinkage products is shown by the impressions they leave on corrosion specimens which are injected under considerable pressure as well as their appearance in distended lungs. The muscularis mucosæ is more developed and the bronchial cartilages are well formed. In general, the relations of the lymphatic system has not changed; lymph glands may be observed forming in the neighborhood of the root of the lung, and large bronchi in pigs as young as 12 cm. They naturally increase in size and number with the age of the embryo. With the other changes, there has been a gradual flattening of the epithelium in the growing ends of the tree, until, in an embryo 18 cm. long, the end buds are lined by a very flat form of cubical cells with spherical nuclei. The cytoplasm, which in the earlier stages was granular, is now clear and transparent.

At 19 cm. (Fig. 26), some notable changes have been inaugurated in the structures. The ciliated epithelium of the stem bronchi possesses a great number of goblet cells. In the submucosa, the muscularis has gathered into distinct bundles, while from the fundus of the crypt-like

invaginations between the mucosal folds appears an ingrowth of glands, containing partly serous cells and partly mucous cells which penetrate sometimes as far as the muscularis and sometimes between its bundles into the submucosa between it and the bronchial cartilages. In general, the relations of the lymphatic system (Fig. 26 *l*) have not changed, but

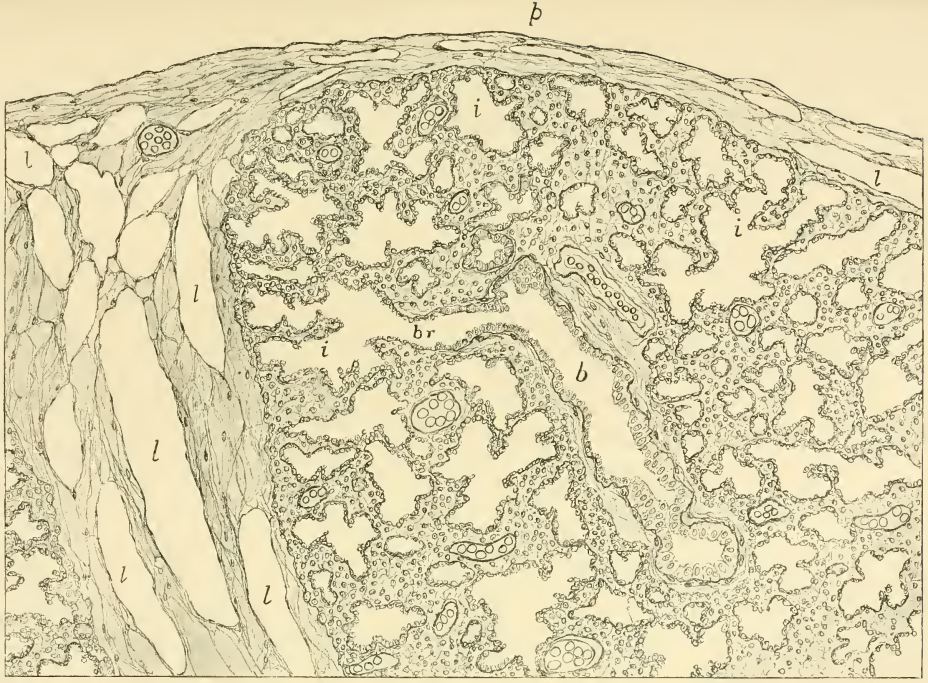


Text Fig. 26.

Text Fig. 26. Lobule of the lung from a pig 19 cm. long. Same preparation as used with the tissue shown in Fig. 21. × 70. *b* = bronchus. *p* = pleura. *l* = lymphatics. *c* = end buds. *a* = connective tissue.

the connective-tissue lobules (Fig. 26) containing the growing ends of the bronchial tree have increased considerably in size. The framework (Fig. 26 *a*) is denser around the end buds (Fig. 26 *c*), which, while still lined by flat cubical epithelium, now show a dilatation of their lumina preparatory to the formation of the respiratory lobules of Miller.

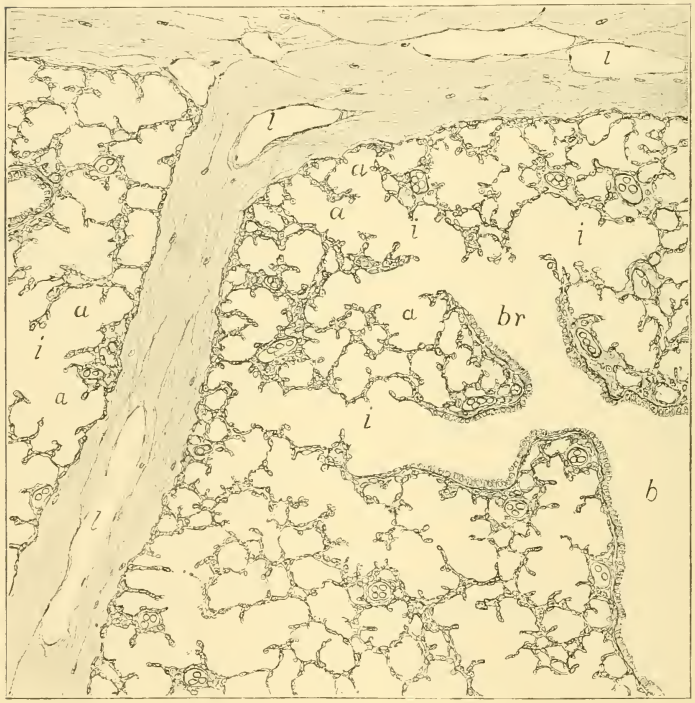
In pigs about 22 cm. long (Fig. 27), the chief changes are in the grow-



Text Fig. 27.

Text Fig. 27. Section of lobule of the lung of a pig 22 cm. long. Same preparation as used with the tissue shown in Fig. 21. × 130. *p* = pleura. *l* = lymphatics. *b* = bronchiolus. *br* = bronchiolus respiratorious. *i* = ductulus alveolaris.

ing end buds (Fig. 27 *i*) which now have an extremely complicated contour and show widely dilated lumina. As they begin to pack together in the lobule, the connective tissue is compressed between them, and its nuclei in consequence appear more numerous. From the low cubical



Text Fig. 28.

Text Fig. 28. Section of a lobule of the lung of an embryo pig 27 cm. long. Same preparation as used with the tissue shown in Fig. 21, except that the lung was distended with the fixing fluid. × 130. *b* = bronchiolus. *br* = bronchiolus respiratorius. *i* = ductulus alveolaris. *a* = atria. *l* = lymphatics.

epithelium of the smaller bronchi (Fig. 27 *b*) the transition is easy to follow over into the irregular flattened epithelium that now lines the young respiratory lobules. The nuclei are pressed against the sides of

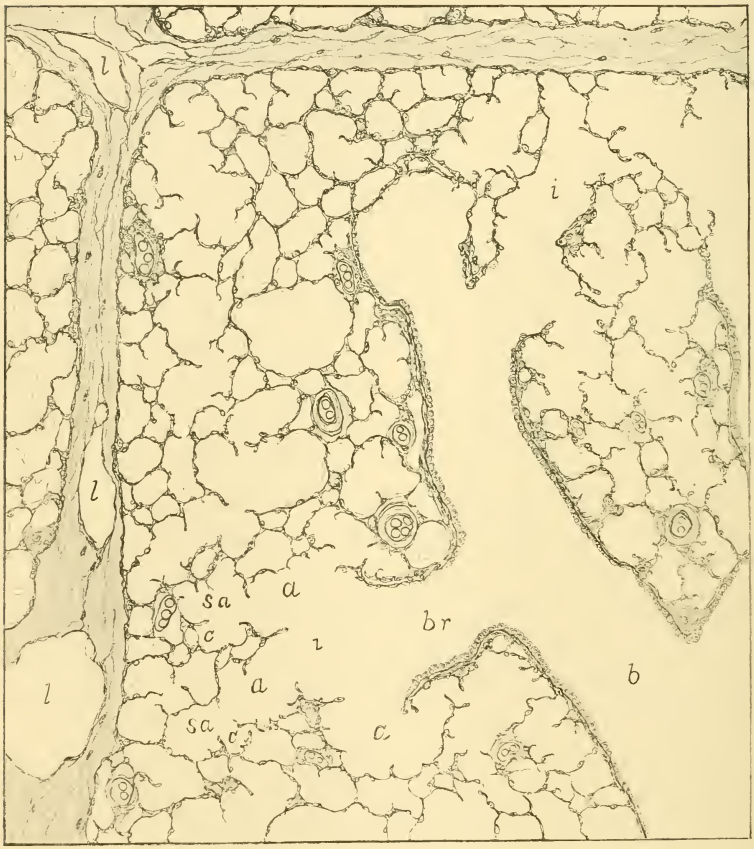
the lobules and the relatively slight amount of clear cytoplasm extends between them. The Bronchioli respiratorii (Fig. 27 *br*) are now readily recognized leading off from the bronchioli (Fig. 27 *b*). They open into the dilated Ductuli alveolares (Fig. 27 *i*) from which the primitive Atria may be seen as lateral outgrowths.

Shortly before birth, in a pig 27 cm. long (Fig. 28), the framework of the lung at the root, between the lobules and under the pleura, consists of definite trabeculæ composed of fibrils in the meshes of which lie the connective-tissue cells. In the neighborhood of the root, the trabeculæ are thick and firm and thin out as the periphery is reached. The structure of the stem bronchi is on the same plan as in the earlier stage, but the epithelium submucosa, muscularis, and cartilages are more developed. As the periphery is approached in this, as in the younger stages, they become essentially younger in structure, loosing first their cartilages, then the muscularis, and finally, before terminating, have only a thickened basement membrane which contains connective-tissue cells (Fig. 28 *b*). The respiratory lobules are now fully formed, but are not as large or as complicated as in the stages after birth. In this section there are two Bronchioli respiratorii (Fig. 28 *br*) from the ends of which the Ductuli alveolares (Fig. 28 *i*) lead. These terminate in dilated Atria (Fig. 28 *a*) on the walls of which the Sacculi alveolares are now indicated as slight irregular outgrowths. While complete corrosions of the lungs in which the respiratory lobules are injected are of great service in interpreting the pictures found in sections, I have feared to trust these preparations for an exact description of the growth of these structures, owing to the possibility of artefacts. The nuclei of the respiratory epithelium now project often into the lumen of the air spaces. In general, the cells are extremely flattened and the nuclei elongated. A flat sheet of protoplasm extends out from either pole of the nucleus resting upon the mebrana propria. Here and there, where capillaries project into the lumen of the air passages, the nucleus lies in the angle formed by the capillary and the basement membrane with the protoplasmic portion of the cell projecting up over the capillary, like a non-nucleated plate.

Adjacent Lobuli respiratorii impinge on each other, pressing the loose connective tissue, which has hitherto existed between the lobules into a thin membrane in which the capillaries run. This interalveolar membrane now consists of the membrana propria of the adjacent lobules, together with the interalveolar connective tissue. The lymphatics in the various parts of the lung still show essentially the same relationships.

After birth (Fig. 29) the development of the lung has advanced along

the same lines followed in embryonic life. The chief changes occur in the respiratory lobules. The bronchiolus (Fig. 29 *b*) is clothed by cubical epithelium surrounded by a well-marked basement membrane



Text Fig. 29. Sections of a portion of the lobule of the lung of a pig, two days old. Same preparation as used with tissue shown in Fig. 28. × 130. *l* = lymphatics. *b* = bronchiolus. *br* = bronchiolus respiratorius. *i* = ductulus alveolaris. *a* = atria. *sa* = sacculi alveolares. *c* = alveoli pulmonaris.

about which are numerous connective-tissue cells. There is as yet, however, no differentiation of this layer into muscle fibers. From this arise the short Bronchioli respiratorii (Fig. 29 *br*) where the cubical epithelium flattens as the passages run into the Ductuli alveolares (Fig. 29*i*). From these structures, the Atria (Fig. 29 *a*) are formed, which in turn produce the Sacculi alveolares (Fig. 29 *sa*). The air sacs which were only indicated in a pig 27 cm. long are now distinctly seen. It is possible that they are even more developed before birth than is shown in Fig. 28, as I have frequently found embryos in utero 29 cm. long. Unfortunately, I have been unable to obtain good sections from specimens of this age. This makes, however, no essential difference as the whole respiratory lobule is produced before the pig is born. Following the use of the lungs for respiration, there is a dilatation of the various structures of the lobule (cf. Figs. 28 and 29) which is accompanied by a still greater flattening of the connective tissue between the alveoli, yielding practically a single membrane containing the blood-vessels between the two layers of respiratory epithelium. This, however, as we have seen, ontogenetically consists of the two basement membranes and the interalveolar framework of the adjacent alveoli. The larger connective-tissue lobules still retain their general relationships, increasing in size with the growth and dilatation of the respiratory lobules of Miller. The lymphatics (Fig. 29 *l*) still have their regular relationships.

In a half-grown pig, one observes the thickening of the framework, which in the main septa at the root and under the pleura is now made up of well-formed trabeculæ, consisting of connective-tissue fibrils. The bronchi have developed peripheralwards taking on an older type, *i. e.*, adding muscular layers, submucous glands, and bronchial cartilages, which may be traced as far as the larger intralobular branches. From this point peripheralwards, gradually thinning, the muscle layer extends to the opening of the atria in the Ductuli alveolares. The lymphatics in the interlobular septa are difficult to see as they are pressed together by the growth and distension of the connective-tissue lobules. No marked changes occur between this and the adult stage, save that the lobules are sometimes less apparent owing to their larger size and the fact that the septa may become thinned out in the later stages of growth. They may be demonstrated as definite anatomical structures in the pig by thick sections stained by Mallory's method or better still by complete Wood's metal injections. When a lung has been distended for a short time with air to its maximum, Wood's metal will pass into all the individual alveoli. After digestion, we have a cast of granular appearance

which maintains absolutely the form of the lungs. This may now be broken up into the lobules, as the splitting always occurs along the septal lines and, thus, the entire connective-tissue lobular system may be revealed. It should be observed that the lobules may become compound through a failure of the septa to persist, a process similar to that which takes place in the submaxillary gland where the whole series of primitive lobes, which are first formed in the embryo and separated by well-marked septa, disappear and are indicated in the adult only by irregular septa, without distinct relationships, passing in from the capsule. Usually, however, these lobules in the pig's lung not only persist, but may be easily demonstrated by any of the ordinary connective-tissue stains.

Recapitulation of Organogenesis.—In recapitulating the growth of the main structures of the lungs, we have stem and main bronchi originating in the primitive lung sacs as an epithelial tube with a double layer of epithelium, the inner of which is columnar, while the outer is composed of smaller polygonal cells. This simple tube is surrounded by a membrana propria formed by a deposit of fibrils from the exoplasm of the connective-tissue syncytium. As the bronchi grow, a layer of spindle cells differentiates from the mesoderm, which is transformed into the muscular coat of the bronchi. Later still, a chondrification of the perimuscular syncytium takes place from which the cartillaginous rings of the trachea and the bronchial cartilages are formed. With these changes the connective-tissue fibrils become grouped into trabeculæ about the bronchi and in the submucosa. Later, the mucosa is thrown into a series of longitudinal folds, while from the cuticular border of the inner row of cells, cilia develop. From the bottom of the crypt-like invaginations formed by the longitudinal folds of epithelium, glands begin to grow down into the submucosa, which sometimes pass between the developing muscle bundles into the deeper layers of this coat. As this process takes place, there is a differentiation of some of the epithelium into goblet cells, a process which also takes place in the glands, giving rise to a series of submucous glands with partly serous and partly mucous cells. While these changes are taking place in the mucosa, the cartilages are also growing, and with them, a further differentiation of the framework into distinct fibrous trabeculæ. As we follow the bronchi peripheralwards, they become simpler and essentially younger in structure and yet develop their adult characteristics in precisely the same way. The epithelium soon becomes single layered of a columnar type, and then of a distinct, flat, cubical form. The Lobuli respiratorii begin to develop in pigs about 19 cm. long by a slight dilatation of the growing ends of the bronchi. These represent the bron-

chioli. Later, the Bronchioli respiratorii are formed which have a progressively flattened epithelium, running over into Ductuli alveolares. These are present at the age represented by a pig 22 cm. long. Subsequently, Atria, Sacculi alveolares, and Alveoli pulmonis form in the prenatal period, all of which have the characteristic flattened respiratory epithelium. After birth there is a dilatation of the lobules and a further flattening of the epithelium occurs, and before the pig is half grown, a muscle layer develops about the air passage as far as the Atria, where it stops in sphincter-like bands.

The framework of the lung develops from a general syncytium forming the mesodermic anlagen of the two lung wings. By a gradual differentiation of connective-tissue fibrils from the exoplasmic part of the syncytium, the framework becomes denser and, finally, at 8 cm., a suggestion of lobulation is obtained about the end branches of the growing bronchi. Within the lobules the framework differentiates as the embryo grows, forming simultaneously basement membranes for the young bronchial buds. At the same time, the interlobular fibers, and those beneath the pleura, unite to produce trabeculæ. As the lobulii respiratorii towards the end of fœtal life begin to impinge on each other, the interalveolar framework and the two adjacent basement membranes are pressed together into a single wall or septum in which the blood-vessels run. These lobules remain until adult life, and correspond in the pig apparently to those described by Laguesse and d'Hardiviller, 98, and Councilman, 01, in the human lung. Noteworthy, however, is the fact that they may become compound by the loss of the interlobular septa and the subsequent confluence of several adjacent lobules. This usually takes place at the base leaving the periphery of the compound lobule separated by partial septa.

The lymphatics appear at the root of the lung in an embryo 4-5 cm. in length. Accompanying the bronchi and vessels, they gradually grow in for some distance and until the smaller air passages are reached, they leave these structures and grow towards the pleura in the interspaces between the smaller bronchi, aiding in the differentiation of the connective-tissue lobules. The reason for this course is not entirely clear, but it may be due to the increasing density of the framework about the bronchi, which forces the later-appearing lymphatics into the interlobular spaces as a Locus minoris resistentiæ. Upon reaching the pleura, they turn and form a plexus in the subpleural connective tissue. Here and there they may be seen penetrating into the lobules, but cannot be followed for any distance in them. At 23 cm. the first evidence of the

submucous lymphatic plexus is seen in the stem bronchi. It may, however, be found earlier, but the vessels are difficult to follow in uninjected specimens.

It would seem, thus, that we have in the pig's lung, besides the lymphatic plexuses accompanying the bronchi, arteries and veins, an interlobular system which Miller has been unable to find in the human lung. Injections pointing to such a relationship he has interpreted as artefacts. If Miller's conclusions prove to be correct, then the lymphatics of the human lung must develop so far as the interlobular septa are concerned in some other way.

In following the organogenesis of the lungs in the pig, one finds at no period in their life history, openings, or fenestræ, which suggest a communication between adjacent respiratory units. They form, as we have seen, independently at the growing ends of the tree and as they approximate each other, it is always possible to demonstrate the interlobular or interalveolar framework without interruptions suggestive of fenestræ offering a communication between adjacent alveoli. Furthermore, in all my corrosions, many of which are complete enough to fill completely the Alveoli pulmonis and maintain the entire form of the lungs, no instance was found of an interalveolar communication. Ruptures frequently occur forming irregular extravasations, but in the most complete injections, one is always able to isolate completely the individual Lobuli respiratorii. The results of this paper, then, support the conclusions of Miller, Laguesse, and Oppel, and are not in accord with the views of Hansemann, Zimmermann, Merkel, and Schulze with reference to the presence of these foramina in the walls of the alveoli of the mammalian lung.

Discussion of the Literature.

the anlage of the lungs.

As in the case of the early stages of the amphibian and reptilian lung, there is a general agreement among most authors who have worked upon the mammalian lung that the respiratory apparatus arises from an unpaired anlage, which the majority regard as asymmetrical. Of these investigators, His thinks the future asymmetry of the lungs is to be sought in this characteristic of the anlage, while Minot looks upon the asymmetry of both anlage and lungs as secondary to changes taking place at this time in the heart. Fol believes the anlage is paired and regards it, moreover, like Götte and Weber and Buvignier as associated with the gill pouches. The anlage, in the pig, arises from the ventral portion of

the head gut as a ventral groove with a more marked projection at the caudal extremity, which becomes separated from the dorsal segment of the gut by two longitudinal fissures, along the line of which the final separation occurs. The upper part of the anlage gives rise to the trachea, the lower to the lungs. If the pulmonary apparatus in mammals should finally be shown to have a serial relationship with the gill pouches, all trace of the process is certainly lost in the pig. From the first, the anlage is asymmetrical. Whether this is a characteristic of the respiratory apparatus or is due, as Minot suggests, to the influence of the heart, it is impossible, from my material, to say. Suggestive, however, is the fact that the pulmonary anlage in many of the lower animals is symmetrical.

THE GROWTH OF THE BRONCHIAL TREE.

Few of the many characteristics of the bronchial tree have given rise to more discussion than the method of its growth. Between the two extremes of dichotomy and monopody, most of the possible intermediate processes have been described. A special review of the literature on this point seems desirable to see what harmony can be drawn from the different observations. So far as possible when space permits, the process will be described in the words of the various contributors to this field.

If we recapitulate the history of the several series of bronchi it may be said that all of the chief bronchi are produced in the same manner, that is to say by monopodial growth. Even the formation of the stem bronchi from the pulmonary anlage does not differ in any material way from the subsequent formation of the products of the stems themselves. As the tree grows, there is no definite division of the end bud as the main branches are outgrowths of the walls of the trachea or the two stem bronchi. In the pig, the trachea produces only a single element, namely, Lateral 1. The process of growth is successive, that is to say, the elements are produced one after another from above downwards, recapitulating the manner of growth shown in simpler animals like the reptiles, for example. When a new element is about to be formed, one notes an increase in the number of karyokinetic figures in the epithelium in the region of the new branch. The basement membrane becomes much less distinct and the connective-tissue nuclei in the surrounding mesoderm are more closely packed together. In this region, a slight bulging of the epithelial wall is then noted, as is shown, for example, in Fig. 12, which increases in size until a small elevation is raised on the surface of the stem. This subsequently grows, yielding a rounded projection on the stem, which gradually emancipates itself and gives rise to a new bronchus.

The process is essentially the same whether it occurs either in the neighborhood of the terminal bud, higher up on the stem, or on the trachea. In general, we may say that the lateral and medial bronchi are produced nearer the terminal end of the main bronchus, while the dorsal and ventral elements are produced somewhat higher up from the stem, often where the latter has regained its cylindrical form.

If Narath's interpretation of the bud as reaching up to the last apparent lateral branch is allowed to stand, then all of the branches except the tracheal bronchus must be considered in the sense of Narath as lateral productions of the end bud. Narath's distinction, however, does not seem to be well made for, in the pig's lung at least between the last lateral branch and the tip of the stem bronchus, there is always a considerable portion of the main stem which has a definite cylindrical form and terminates in a distinct dilatation at the end. Much as Narath's view would tend to simplify the question, there is little justification, therefore, in looking upon the entire distal part of the stem bronchus as the terminal bud. On the other hand, there is no essential difference in an evagination taking place at the bud and in one taking place on the stem.

It may be well to notice certain differences in the behavior of the stem at different periods in the life of the organism as well as differences between different species. For example, in the pig, the stems seem relatively more irregular and dilated in size in embryos between 10 and 13 mm. long, but on the whole are fairly cylindrical throughout the growing period. On the other hand, in some species the stems, particularly at the growing ends, are quite irregular in shape and may be considerably dilated, suggesting somewhat pictures corresponding to the growing lungs of reptiles.

After the formation of the chief branches has occurred, the primitive monopodial system may persist for a few generations on the side branches. The principal method of division is, however, by dichotomy equal and unequal. Apparently the selection of the method depends somewhat on the physical conditions of the space in which the bronchi are forced to divide. In the case of the first divisions of Lateral 1, of Lateral 2 on each side, and Ventral 2 on the right side, the division is of practically equal dichotomy, as they have a relatively free space about them. When, however, the direction is more or less controlled by the limited environment of the bronchi, it becomes unequal, one fork growing on so rapidly to become the stem, that the other is left either as a small bud or a small side branch, which develops further when the space relations permit.

Later still when the total volume of the lung is such that each bronchus is more or less equally surrounded by mesoderm, the dichotomy is equal, although of the two forks resulting from a division, one becomes the stem and the other is shunted off as a side branch. The point, however, where monopody ceases and dichotomy begins is apparently different in different species and may be different in different parts of the lung. In the pig it is below Lateral 6 while, in man, according to His, the transfer is made at Lateral 4. It must be remembered in this connection, however, that the space relations in this region of the human lung are quite different from those in the pig owing to the different position of the heart, diaphragm, and liver.

The bronchi, apparently, show great adaptability both in the power and direction of their growth. This interesting characteristic is best shown when one of the chief bronchi are suppressed. Adjacent branches, while still rooted firmly at their point of origin, then grow into the area of the lung usually supplied by the suppressed element, a process which, taken in connection with the extreme variation of the point of origin of the bronchi, give rise, in the adult tree, to the series of pictures which suggest a wandering of the branches. In my whole series of specimens numbering ten reconstructions and many cleared specimens 3 to 18.5 mm., and about 100 corrosions of pigs from 4 cm. to the half-grown stage, I have never found any evidence which pointed to a wandering of any elements of the tree. The bronchi remain attached to their stems where they are formed, although their branching is controlled to a great extent by the space in which they have to grow. When this is altered by the suppression of one of the usual elements, adjacent branches show a power of substitution which is perhaps best exemplified in the fate of the two dorsal forks of the first division of the right and left Lateral 2. On the right side, this branch, owing to the presence of the Lateral 1 above it, is forced to grow downwards and posterior to form a dorso-inferior branch of Lateral 2, while on the left side, this same fork, unobstructed by the absence of Lateral 1, grows upwards to substitute for the suppression of the lateral element above.

In turning to the literature we find that between such outspoken descriptions as those of d'Hardiviller for example, on the one hand, and Justesen, on the other, it is not difficult to differentiate, but in the cases where terms like sympodial dichotomy and monopody with acropetal development of the lateral buds are used, it is not always easy to determine whether the authors have not been describing the same process with different words. At the outset, therefore, it may be well to state that

by monopody we understand lateral outgrowths from the wall of the bronchus whether they occur on the side of or above the terminal bud, and by dichotomy we understand an undoubted division of the terminal bud. In equal dichotomy the two divisions grow for a time equally but later may give rise to a system of monopodial appearance by the selection of one branch to continue as the stem, while in unequal dichotomy the two buds develop unequally from the first. In the case of dichotomous divisions, however, it is obvious the portion of the stem between two side branches is genetically equivalent to the side branch of the lower order.

Since one can explain theoretically the entire bronchial tree equally well by either a monopodial or a dichotomous process of growth, it is not surprising to find different views among those who have studied only the finished bronchial system. This is well shown among modern investigators in the work of Aeby, 80, and Ewart, 89, the former of whom believed in monopodial growth from first to last, while the latter says "Dichotomy is the alpha and omega of bronchial division." Huntington, 98, also in working upon comparative material of adult stages finds a double system primarily dichotomous with a subsequent monopodial type of branching in the development of the stem bronchus. In a system thus capable of two explanations, obviously, the only observations which will really aid in solving the question come from those who have studied the lungs during the process of their growth.

If we turn to this series of investigations we find Küttner, 76, stating that "Das Wachsen ist monopodisch, d. h. das Epithelrohr wächst an seinem Scheitel ungetheilt fort, während seitliche Sprossen am Stamm desselben hervortreten und mit ihrer Längsaxe zu der des erzeugenden Rohres rechtwinkelig gestellt sind." Furthermore, he states that these buds grow and divide rapidly, giving rise to so many more lateral branches than the principal axis that it is difficult in the adult tree to recognize its primitive monopodial character.

Cadiat, 77, describes the process as follows, and it is important to remember he is speaking of solid buds: "Il est facile de comprendre maintenant comment se produisent les ramifications bronchiques. Un premier bourgeon se forme plein et se développe en longueur, l'ampoule se produit à l'extrémité. Alors son evolution est arrêtée; sur les parois naissent des bourgeons secondaires qui se terminent de même, et ainsi les canaux bronchiques vont sans cesse en se multipliant, mais toujours dans des directions différentes."

Stieda, 78, states: "Zuerst ist der Canal einfach, dann theilt er sich

in Aeste, welche sich abermals theilen, so dass sowohl durch fortgesetzte Theilung des auch durch seitliche Sprossenbildung im epithelialen anfangs noch leicht übersehbaren Canalsystem ensteht, dessen blinde Enden etwas leicht erweitert sind."

Kölliker, 79, describing a 12-day rabbit embryo, says: "Das innere Epithelialrohr, das nun Bronchus heissen kann, hat in jeder Lunge drei Ausbuchtungen und werden von nun an mit dem Grösserwerden des Organes die Verästelungen bald so zahlreich, dass dieselben nur schwer Schritt für Schritt zu verfolgen sind." Further, in speaking of the increase of the bronchi in man and animals, he says in general: "Das innere Epithelialrohr hohle Aussackungenen oder Knospen erzeugt, welche, rasch sich vermehrend, bald in jeder Lunge ein ganzes Bäumchen von hohlen Kanälen mit kolbig ausgeschwollenen Enden erzeugen, von welchen aus dann durch Bildung immer neuer und zahlreicher hohler Knospen endlich das ganze respiratorische Höhlensystem geliefert wird." His, 87, in working on the development of the human lung, describes the process of growth as follows: The first branches as far as Lateral 4 arise by monopodial division, which he describes in the following terms: "An keiner Stelle findet sich eine Andeutung, als ob aus den einmal cylindrisch gewordenen Wurzelröhren Seitensprossen zu entstehen vermöchten. Die einzige Productionstätte neuer Formbestandtheile sind die Endknospen, und zwar erfolgt die Umgestaltung auf dem Wege dichotomischer Theilung. Die Knopsen verlieren ihre kugelige Grundform, indem sie an der Anheftung gegenüberliegenden Seite sich abplatten und zugleich in transversalem Sinne strecken. Bald tritt eine trennende Furche auf, wodurch die ursprüngliche einfache Knospe in zwei getrennte Verwölbungen auseinander geht. Allmählich emanzipiren sich diese letzteren und bekommen auch ihrerseits cylindrische Stiele, worauf-hin derselbe vorgang von Neuem Platz greifen kann." In summarizing the process he continues: "Nach erfolgter Trennung der beiderseitigen Anlagen bildet eine jede derselben einen gebogenen und zugleich birnförmig ausgeweiterten Schlauch, mit einzelnen schärfer markirten Vortreibungen. Aus diesen treten die primären Seitensprossen als monopodische Bildungen im Sinne von Aeby hervor und ihre für beide Seiten asymmetrische Anlage bestimmt auch die Differenzen späterer Ausbildung. Der weitere Verzweigungsmodus bleibt nun während geraumer Zeit der dichotomische. Zuletzt tritt aber ein Zeitpunkt ein, wo die Endknospen aufhören sich dichotomisch zu theilen und wo sie wieder in ein System mehr oder minder ausgiebiger Seitenknospen auslaufen."

In mouse, mole, and pig, Willach, 88, describes the process as follows:

" Ich glaube vielmehr, dass beim Menchen, wie bei den Säugethieren, die Sprossung eine sogen monopodische ist, welche darauf beruht, dass das Mutterrohr vor seinem kugeligen Endbläschen eine Verengerung seines Lumens erfährt, während das Lumen des Endbläschens sich erweitert und seitliche Ausbuchtungen treibt, jene Knospen, die wieder zu Röhren werden, und das Mutterrohr weiter fortwächst. Das Tochterrohr ist enger als das Mutterohr."

The growth process is described by Robinson, 89, in these words: " In the rat and the mouse, the ramification of the bronchi is produced principally by dichotomy. The germ of each bronchus, as it grows outwards and dorsally, becomes expanded at its termination; this expansion is gradually constricted into two portions of unequal size, that is the dichotomy is in the form described by botanists as unequal or sympodial." Further he states: " Although most of the branches are produced by dichotomous division of terminal expansion, certain of the dorsal branches arise as hollow buds from the wall of the stem bronchus after it has assumed its cylindrical form, and these buds are interpolated between preexistent branches." He describes the origin of our median bronchi in the rat as follows: " The second dorsal branch immediately after its origin is similarly divided, and the constriction passes rapidly towards the axial stem, until its apex reaches the level of the circumference of the main bronchus. Thus, from the dorsal bud, a dorso-internal (median) branch is formed." Robinson apparently does not believe that the branches are successive in their formation.

Minot, 92, states that " the branching occurs in a highly characteristic manner, for the stem always forks, but the forks develop unequally, one (terminal bud) growing more rapidly and becoming practically the continuation of the main stem, while the other (lateral bud) appears as a lateral branch. Speaking in general it may be said that the ventral fork serves as the stem. In consequence of this method of growth the adult lung consists of main stems with lateral branches. . . . But it is erroneous to suppose, as did Aeby, that the system of growth is strictly monopodial, it being in reality a modified dichotomous system. The branches all arise by terminal forking, never as outgrowths from the side of a stem."

d'Hardiviller, from his studies on the rabbit and sheep, announces the following law of development: " Toutes les bronches primaires, principales ou accessoires, naissent en divers points des bronches souches par ramification collatérale, le bourgeon terminal des bronches souches ne prenant aucune part à leur formation." These principal branches then,

according to d'Hardiviller, give rise to secondary branches by the production of lateral buds as well as by equal and unequal dichotomy. d'Hardiviller does not believe that all branches of the stem are successive in their formation.

Nicholas and Dimitrova, 97, in the sheep, describe the growth of the main bronchi as lateral buds which appear successively on the terminal portion of the stem bronchus.

The results of Justesen, 00, contained in an extensive paper devoted entirely to the method of growth of the bronchial tree, may be given in one sentence, "Die Bronchialverzweigung ist also eine dichotomische," in which process he would include all branches of the tree from first to last.

The process of growth of the bronchial tree according to Narath, 92, 96, 01, is a rather complicated process. He looks upon the primitive lung sac as the first production of a stem bud. When a side branch is produced from the end bud a slight swelling is observed on its lateral side, emphasized by the occurrence of mitosis in this region. In consequence of the greater pressure at this point, the end bud bends slightly in the opposite direction, that is to say, medialwards. As the new bud grows, this process continues until there is a distinct kink in the axis of the stem opposite the new element. As it increases in size, the side bud takes first, the form of a cone-like projection with a rounded summit, as the stem bud grows on, then the epithelial wall about its base sinks somewhat towards the axis of the stem, until the daughter bud is isolated from the stem and then grows on. It is important to note, furthermore, that Narath considers the end bud the entire terminal part of the stem up to the last well-formed lateral branch.

In reference to the origin of the dorsal bronchi, Narath states from his observations on the rabbit, that they are produced without participation of the stem bud and that they appear later than the corresponding lateral bronchi. Furthermore, the comparative anatomy of the tree suggests to him that the dorsal series are primarily side branches of the lateral bronchi which, in course of ontogeny or phylogeny are placed back on the stem. In support of this view, he finds the dorsal buds arising at the same level as the lateral and, apparently, in communication with the contour of the latter. Then, he continues, if lateral bronchi are able to give up dorsal branches to the stem, this process repeats itself with the latter series in giving rise to the median bronchi. While he is not absolutely certain that this process takes place in the origin of the dorsal elements, he states that it can be proved with certainty in the formation of the medial series. He shows a schematic series of draw-

ings of the median branches of D. 2, D. 3, and D. 4 in their different stages, giving an apparent transplantation of this median branch upon the stem bronchus. Like the median series, Narath also believes that the ventral bronchi (the Ventro-accessory of Aeby) are branches which are given up from the lateral branches to the stem. In one rabbit embryo Narath was able to show a relationship between Ventral 1, the infracardiac bronchus, and Lateral 1. He says further: "Der Zusammenhang der Knospen ist ein primäres Verhältnis und kein sekundäres. Und wenn weiter eingewendet werden sollte, die Knospen hängen deswegen so innig zusammen, wei bei der erwachsenen Lunge die Bronchien so enge beisammenstehen, so würde ich auch wiederum gerade diesen Befund bei der erwachsenen Lunge als für die Aeby'sche Ansicht sprechend verwerthen." In a word, while not absolutely pledging himself to this view, Narath believes that there are but one primary set of bronchi, namely the lateral, and that the other three series, the dorsal, ventral, and medial originate either directly from these branches as in the case of the dorsal and ventral groups, or the median branches of the dorsal series as in the case of the median bronchi, and are then given up on to the stem bronchus.

Moser, **02**, says for the vertebrate lung in general that "Das Verzweigungssystem der Kanäle innerhalb der Lunge ist stets und ausschliesslich ein monopodiales." It must be remembered, however, that Moser's material on the mammalian lung was very limited and confined to older embryos which were studied by means of sections instead of corrosions and reconstructions. Some criticism might be made of her comparative material especially in view of the more exact methods used by Hesser in the same field.

Blisnianskaja, **05**, in the human lung states that "Die Bronchialverzweigung geschieht nacht dem dichotomischen Typus, der durch ungleiches Wachstum der Gabeläste ein monopodisches Aussehen erhält."

Hesser, **05**, in his important work on the reptilian lung states that "ausser allem Zweifel, bei niederen wie bei höheren Reptilien die erste Äste aus dem Stammbronchus monopodial angelegt werden. Tarentola, Anguis, Chrysemys u. a. zeigen dies unzweideutig. Die Bronchien haben eine ansehnliche Länge erreicht, bevor noch Seitenäste auftreten, und wenn die erste Knospe sichtbar wird, tritt sie aus der Seite des Bronchus hervor, und zwar in einer bedeutenden Entfernung von dessen kaudalem Ende." In speaking of the further growth of the branches, he continues, "Denn dadurch, dass das Längenwachstum der Äste nicht porportional zur Vermehrung der Anzahl ihrer Knospen ist, geht die Monopodie allmählich in Dichotomie über. . . . Also besteht zwischen Monopodie und

Dichotomie nur ein gradueller, aber kein wesentlicher Unterschied, und es würde daher kein Erstaunen hervorrufen dürfen, wenn in der Architektur des Bronchialbaumes sowohl die eine wie die andere Weise zur Anwendung gekommen ist."

If we attempt to tabulate these views on the growth of the bronchial tree, the results may be placed in three main divisions as follows:

1. Dichotomy. Older authors, Ewart, Minot, Justesen, Blisnianskaja.

2. Monopody. Küttner, Cadiat, Kölliker, Aeby, Nicholas and Dimitrova, Willach, Narath, Moser.

3. Monopody and Dichotomy. Stieda, His, Robinson, Huntington, d'Hardiviller, Hesser, Flint.

It is also possible to subdivide them still further in the following way:

1. Dichotomy. Older authors, Ewart, Justesen, Minot.

2. Unequal Dichotomy. Robinson (?), Blisnianskaja.

3. Monopody. Aeby, Moser.

4. Monopody with participation of the end bud. Willach, Narath, Nicholas, and Dimitrova.

5. Mixed Monopody and Dichotomy simultaneously. Stieda, Robinson.

6. Monopody and Dichotomy successively. His, d'Hardiviller, Huntington, Hesser, Flint.

While we have already called attention to those who have only studied the branching from the finished tree, to which class belong Aeby, Ewart, and Huntington, there is still a group, in the series of authors given above, who have not followed the lungs through the development of the stem and its chief branches in mammals, that is to say, their material consisted of embryonic stages after the formation of the principal bronchi was complete. The observations of these investigators are only important for the specific fields in which they worked, for it goes without saying, as His has suggestively remarked, the conditions which govern the form development of a growing part need not necessarily remain the same through the different phases of its evolution. It may change its character either once or more than once.

Thus for a series of animals covering amphibia, reptilia, birds (Moser, Hesser, Schmalhausen), man (His), rats and mice (Robinson), mouse, mole (Willach), rabbit (d'Hardiviller), sheep (Nicholas and Dimitrova), rabbit, Echidna, cat (Narath), pig (Flint), we have a general agreement, that the stem and its principal branches are produced by monopodial growth. I have placed Robinson in this group, partly because he believes some of the chief branches are monopodial in nature, but largely because, notwithstanding his own use of the term "sympodial

dichotomy," his own description of the process of division appears to me to be esentially of a monopodial character. Against these views we have the outspoken description of Minot for dichotomy, in the human lung, as well as that of Blisnianskaja. The latter does not describe the process in detail and her illustrations appear to me to be capable of a monopodial interpretation, especially in view of the careful work of His on the same material. It is also noteworthy that she quotes the statements of Justesen in supporting her ideas on the sympodial development of the chief divisions of the stem. It may be recalled, however, that this author did not possess in his material stages which showed the development of these particular branches.

While it is possible to draw much harmony from the verbal descriptions of the process of division which I have given above, there are, of course, many exceptions and different complexions to these views. Since, in my opinion, it makes little difference whether the monopodial outgrowths take place from the end bud or from the stem a little higher up, we may justifiably say that among those who have studied the production of the chief bronchi of the vertebrate lung, the following stand for an absolute monopodial system: Moser, Hesser, Schmalhausen, His, Wilach, Robinson (?), d'Hardiviller, Nicholas and Dimitrova, Narath, and Flint. This series includes obviously all who have worked on the development of the lung during this period except Minot, Blisnianskaja, and Robinson, whom I have placed in both lists. Of these authors, Willach, Narath, Minot, and Blisnianskaja believe that our Lateral 1, the so-called "Eparterial or tracheal bronchus," is a derivation of our Lateral 2, which wanders up on the stem bronchus or trachea, the others look upon it as an independent and unpaired element. Narath and Blisnianskaja regard the other chief bronchi as secondary derivatives of the lateral group as "accessory" in the sense of Aeby. Willach believes the ventral and median groups as accessory, that is to say, derived from the lateral and dorsal bronchi respectively, while Robinson thinks the chief bronchus of the ventral series, Ventral 2 (the Bronchus infracardiacas) is ontogenetically independent, but phylogenetically accessory. The latter describes the origin of the medial bronchi, his dorsointernal group, from the dorsal by a process of progressive splitting of the first medial branch of the dorsal bronchi until it comes to have an independent origin on the stem, a view which is advanced in greater detail by Narath.

All of the arguments of Narath and Blisnianskaja concerning the derivation of the ventral, dorsal, and medial series either primarily or secondarily from the lateral bronchi are quite unconvincing, for like

the support, which Narath brings from the comparative anatomy, the facts are capable of a simpler explanation, *i. e.*, a wide variation in the position of the buds and the power of one bronchus substituting for another. These two factors which I have followed in detail in the pig's lung, will explain all of the conditions in the adult tree which led first Aeby and then Narath and their followers to look upon the ventral and medial groups as derivatives of the lateral series. It may also be well to call attention to Hesser's pointed criticism of Narath's view when he remarks that the lateral buds of Narath when they have only reached the development of a low round cone with a broad base, represent the anlagen of four different branches, namely the dorsal, lateral, ventral, and medial bronchi which must isolate themselves and take their places on the stem. And lastly, we cannot help noting the lack of the one convincing argument which should come from comparative anatomy consisting in a primitive lung that possessed only lateral bronchi.

Furthermore, the series of schematic figures, which Narath gives to show the origin of the medial from the dorsal bronchi are objectively correct and agree with the conditions found in the pig's lung not only in the embryonic stages but in the adult tree as well. He finds the first median division of the dorsal bronchi as one descends from D. 2 to D. 5, is placed successively nearer the stem bronchus until, at the latter point buds are seen on the dorsal and medial sides of the stem. He interprets this condition as indicating a wandering of this median branch to the stem. As a matter of fact, however, this is the normal relationship for the grown lung, and, as I have pointed out above, the medial series do not occur higher than Lateral 4. It is scarcely justifiable, therefore, to interpret the successive change in the insertion of this median branch, together with the appearance of the medial buds in their usual position as evidence of wandering on the part of the median bronchi.

In reference to the further division of the tree after the principal branches are laid down, Moser, Willach, Narath, Cadiat, Küttner, and Kölliker believe in a monopodial propagation, while His, Minot, d'Hardiviller, Hesser, and Flint believe in the dichotomous form either equal, unequal, or both.

AEBY'S EP- AND HYPARTERIAL THEORY.

The substance of Aeby's views with reference to the influence of the pulmonary artery upon the bronchial tree has been given in the abstract of his monograph. This theory, which has influenced, more or less, the work of all subsequent investigators has been accorded a varied reception.

His, Willach, Robinson, d'Hardiviller, and Miller, either actively or passively, support the views of Aeby, while Ewart, Zumstein, Narath, Minot, Huntington, Justesen, and Merkel have abandoned them. In some cases it is difficult to ascertain just what position an author takes concerning the theory for some of them use indiscriminately the terms hyparterial and eparterial in describing the tree. These terms, of course, may have only a simple topographical significance, as in the case with Huntington, without implying the meaning which Aeby attaches to them. Of all the authors who are considered as supporting Aeby's theory His, alone, is outspoken in his belief that the eparterial bronchus is a dorsoventral bronchus which if it were in the hyparterial region would divide into dorsal and ventral branches. Willach, who first describes the eparterial branch as arising from the first ventral bronchus, apparently accepts the theory, although Narath, a few years later advocating the same view, states that this single fact is sufficient to disprove Aeby's hypothesis once and for all. Zumstein attacked the theory from another point of view, namely, by failing to find in corrosion specimens the relationship, which Aeby describes, and by noting variations in the pulmonary artery which, apparently, had no influence on the architecture of the tree. In these observations Zumstein is supported by Narath, who also describes such specimens. Both observers also call attention to the fact that, at the time the primitive bronchi are formed, the pulmonary artery is a fine, delicate vessel which would have no influence on the larger, firmer epithelial structures. Huntington attacks the theory from another point of view in looking upon the wandering of bronchi as the chief factor in the formation of the eparterial bronchi to which the relationship of the artery is simply secondary and topographical.

From the results recorded in this paper, it would appear that the relationship of the arteries to the tree and the differentiation of two sets of bronchi with different relationships to the pulmonary arteries are primarily due to the topography of the anlage with respect to the Vena pulmonalis and the projection of the anlage ventralwards from the head gut. In consequence, the arteries form behind the primitive stems before any of the side branches are produced. Later the first lateral bronchus develops above and behind the artery, while the remainder of the series are formed below and in front of it. As the heart descends, the topography of the arteries to the stems changes, but in no way and at no time have the arteries a fundamental influence in differentiating two segments of the

tree. On account of the association of this influence with the terms "eparterial" and "hyparterial" it is, perhaps, well to abandon them as Zumstein and Narath have suggested. However, this much is certain: The theory ought not to be abandoned without an acknowledgment of our indebtedness to it. That the theory would stand or fall from the results of embryological research, Aeby clearly recognized, much more clearly apparently than some of his critics. As a working hypothesis, his view was generally accepted from the time of its publication until the appearance of Narath's paper.

1st lateral bronchus. "eparterial" bronchus of Aeby.

This, Aeby regards, as a dorsoventral bronchus which lies above the pulmonary artery and, therefore, not under its influence. If it were in the hyparterial region the artery would divide it into dorsal and ventral bronchi, a view in which Aeby is supported by His. It is an independent structure; it may be either paired or suppressed. These characteristics form the basis of Aeby's classification of the mammalian lungs. Willach first proposed the idea that this was a branch of the 1st ventral bronchus, while Robinson, like His, believes it is an unpaired and independent branch. Zumstein in abandoning the eparterial theory terms this the first lateral bronchus. Narath uses the expression Apical bronchus and takes the same view as Willach inasmuch as he considers it a branch of the 1st ventral bronchus. The former, however, goes further in regarding this element as a definite dorsal bronchus. This is compatible with his tentative view of the whole series of dorsal bronchi arising probably primarily from the ventral group. Minot supports Willach, while d'Hardiviller thinks it is an independent element arising from the trachea in sheep and the stem bronchus in rabbits in which view he is upheld by Nicholas and Dimitrova so far as his observations in the sheep are concerned. Justesen, Merkel, and Blisnianskaja follow Willach. The unique and remarkable observation of d'Hardiviller, who states that in the rabbit there exists primitively an eparterial bronchus on each side, is the only suggestive evidence of the degeneration of an eparterial bronchus taking place during the ontogeny of the embryo. For a time each develops symmetrically and then later the left atrophies and disappears. Upon this observation d'Hardiviller concludes that Aeby, His, Robinson, Narath, Nicholas, and Dimitrova are mistaken in stating no bronchus arises at this level on the left side, and, believes in consequence, Aeby's classification of mammalian lungs is only of secondary value. In certain species they may both develop,

in others the left only may atrophy, while in still others both may undergo the atrophic changes leaving the tree consisting only of a symmetrical hyparterial system. This observation of d'Hardiviller has only received a single supporting observation in the whole literature and that is by Bremer in the opossum lung. Bremer finds in embryos of 12.5 mm. what he calls an eparterial bronchus on the left side. In his specimens, 14 cm. long, this is absent and, therefore, he presumes the bronchus has degenerated between the two stages he has been able to observe. Narath, in the possession of two adult rabbit lungs with left eparterial bronchi as variations, is inclined to believe d'Hardiviller is dealing with an abnormality, and, furthermore, in view of the unique nature of the observation, adds that absolutely indisputable histological preparations must be produced to show the degeneration of a bronchial bud which has once been formed. This criticism of Narath would, in part, apply to Bremer's observation. The production of the bronchial tree in Echidna, according to Narath, follows the same principles which we observe in other mammals and the lung of the adult is not differentiated from that of placentalia. Moreover, the vessels and their relationships undergo no further changes while the young are in the pouch either in respect to the artery or the veins. It is thus hardly possible in these observations of d'Hardiviller and Bremer that we are dealing with a true regressive process. In fact, it is more probable that in both cases we are either dealing with a variation or a dorsal bronchus which is placed higher up than usual upon the stem bronchus. This assumption is made quite probable by Bremer's statement that his left eparterial bronchus did not supply the apex of the lung.

This bronchus is undoubtedly one of the lateral series as Zumstein and Nicholas and Dimitrova hold. It, like the remainder of the lateral series, originates from the lateral wall of the trachea or the stem. The fact that it is usually unpaired and has a different topography to the pulmonary artery does not separate it from this group. It is true, the bronchus originates a little more dorsalwards than the remainder of the series, but this is due partly to the different space relationships in the upper part of the thorax and partly, to the ventral torsion of the lower lateral bronchi, which exaggerates the slight difference that occurs between Lateral 1 and the remainder of the series in the embryo.

Inasmuch as a bronchus corresponding to Lateral 1 has never been described in Reptilia or Amphibia, it must be regarded as peculiar to mammals. The great rarity in the occurrence of paired first lateral bronchi suggests that no more morphological significance can be laid on

its presence on both sides than its absence. The unpaired Lateral 1 on the right side must be regarded as the normal condition for mammalia, due to a phylogenetic provision for the descent of the heart and great vessels through the suppression of the element on the left side. In cases where it is formed bilaterally, no instance of a left Lateral 1 on the trachea has yet been described. As Narath shows, it is always somewhat lower on the left side than the right when the element is bilaterally present. From Narath's tables, the bronchus is unpaired on the right side in 199 species, is bilateral in 15 species, and is absent on both sides regularly in 3 species. These three types, apparently, obey no definite law; in the same order of animals, all three types may be found in nearly related species.

In some instances, Lateral 1 arises from the trachea, in others from the stem bronchus. When, however, we observe the conditions in those animals where it is formed on the trachea, we find the bifurcation occurs near the second pair of lateral bronchi. On the other hand, where Lateral 1 is produced on the stem, the division of the trachea takes place high up, throwing it on to the main bronchus. Its dorsal character, in which Narath believes, is, however, secondary, as its lower branches are forced backwards by the presence of L. 2 below it and the relatively free space beside the vertebral column just above the dorsal bronchi.

APICAL BRONCHUS OF WILLACH AND NARATH.

So general is the acceptance of the view that Narath is the author of this idea, it may be well to quote his own words in which he gives the credit to Willach: "Ich bin ganz der Meinung Willach's, dass der apicale Bronchus ursprunglich ein Seitenast des 1. ventralbronchus sei, der auf den Stammbronchus gerückt ist." Willach explains himself thus: "Man könnte also den von Aeby als eparteriell bezeichneten Bronchus als Nebenbronchus zum ersten Ventralen derselben Seite im Sinne Aeby's auffassen, der, wenn bronchial, an den Stammbronchus, wenn tracheal, an die Trachea abgegeben worden ist." Further, he says: "Andrerseits dürfte aber der erste ventral Seitenbronchus der linken Seiten dem der rechten plus dem eparteriellen Bronchus entsprechen. Der erste linke ventralbronchus zeigt nämlich einen nach vorwärts strebenden Ast, der in seiner Gestalt nicht allein Aenlichkeit aufweist mit dem eparteriellen Bronchus bei verschiedenen Thieren; sondern er ist auch geradezu in einem eparteriellen Gebiet gelegen, wenn man von einem ähnlichen, aber doch etwas veränderten Gesichtspunkte aus, als es Aeby

gethan, zwischen dem eparteriellen und hyparteriellen Bronchialgebiet unterscheidet."

Aeby looked upon this apical branch of the 1st lateral on the left side as a simple side branch, which extends up into the apex of the lung having a certain outward similarity to Lateral 1, which might, he pointedly remarks, lead to erroneous assumptions. This branch was named by His, the Bronchus ascendens, an element, which substitutes in the left lung for the unpaired eparterial bronchus in the right, a view in which he is supported by Robinson. Narath and Willach, on the other hand, as stated above, look upon it as the equivalent of the eparterial bronchus, a homology which is affirmed by Minot, Huntington, Merkel, and Blisnianskaja, but d'Hardiviller, and Nicholas and Dimitrova accept the conclusions of Aeby, His, and Robinson. That is to say, d'Hardiviller accepts them in so far as they regard the left apical bronchus of Narath, a true side branch of the 2d lateral trunk and not the equivalent of the eparterial bronchus on the right side.

In following, step by step, the appearance of the secondary divisions of Lateral 2, in the pig, we find on the right side the dorsal fork is turned downwards and outwards owing to the presence of Lateral 1 above it, in consequence of which, it becomes the large dorsoinferior branch of L. 2. On the right side, however, this unobstructed branch extends upwards toward the apex of the lung and substitutes, as Aeby and His pointed out, for the suppression of left L. 1. It is, however, a true side branch of Lateral 2 and is not to be regarded as the homologue of right Lateral 1, which in the vast majority of cases is unpaired.

LATERAL BRONCHI.

Kölliker, who worked on the rabbit, agrees with the observations of Remak on the chick in finding the first branches of the stem bronchus growing lateralwards and dorsalwards. He did not, however, give the lateral group a special name. Aeby, whose observations were made upon full-grown material, designated them ventral bronchi in contradistinction to the dorsal group, both of which arise in the hyparterial region from independent origins, while in the eparterial region the dorsoventral bronchus uninfluenced by the pulmonary artery has a common origin from a point on the stem bronchus or trachea midway between the origin of the dorsal and ventral bronchi in the hyparterial group. Although His would have preferred the term lateral bronchi, he follows the description of Aeby, while Robinson is really the first to take his term lateral bronchi from the topography of the embryonic lung. Zumstein

and Nicholas and Dimitrova have accepted Robinson's terminology, while Willach, Narath, Merkel, and Bremer have followed Aeby. Although he believes the selection an unhappy one, Narath, like His, uses the term "ventral" simply because it has received general acceptance in the literature and because the bronchi run to the ventral part of the lung. All of the lateral group receive a topographical nomenclature from Ewart, while d'Hardiviller calls them "external bronchi," and Blisnianskaja "the ventrolateral" group. Curiously enough, these are the only branches of the entire bronchial tree which all authors unanimously agree, despite the different terminology, are wholly independent derivatives of the stem bronchus.

Owing to the topography of the origin of this series of bronchi from the lateral wall of the stem, the author has followed Robinson, Zumstein, and Nicolas and Dimitrova in their nomenclature instead of Aeby and His. This is quite logical for, as His has pointed out, all of the ventral characteristics of this group are secondary to their later growth ventralwards in the space between the diaphragm and chest wall. The spiral line formed by joining the origins of the lateral bronchi on the stem represents the extent of ventral growth of these bronchi, as the upper elements reach farther ventralwards than the lower and consequently the torsion of the stem is greatest above and gradually diminishes as the lower elements are reached. These occupy practically the lateral plane of their origin. Finally, the presence of a real set of ventral bronchi in many species renders the change in the nomenclature urgent.

DORSAL BRONCHI.

With the exception of Ewart, d'Hardiviller, and Blisnianskaja, all authors designate this group the dorsal bronchi. d'Hardiviller calls them posterior bronchi, while the latter classifies them as a dorsolateral group. There is also a general agreement that they are independent derivations of the stem bronchus, although Narath, without absolutely pledging himself to this view, is inclined to look upon them as a group primarily derived from the lateral series. He reaches this conviction partly because he regards the "Eparterial" bronchus as the first dorsal bronchus and a definite dorsal branch of Lateral 1 and partly because they bear a certain similarity to branches of the lateral group. In consequence of the shifting of his Dorsal 1 up on to the trachea or stem bronchus, Narath regards Aeby's D. 1, D. 2, D. 3, etc., as D. 2, D. 3, and D. 4, respectively. In looking upon the dorsal group as derivatives

of the lateral bronchi, Narath has the support of Blisnianskaja, who argues if the "eparterial" is a dorsolateral bronchus, it is reasonable to suppose the remainder of the series are similarly derived. Neither of these authors, however, have followed the wandering step-by-step either of the eparterial or the dorsal branches on to the stem bronchus. They are, on the contrary, independent derivatives of the stem and, like the lateral series, are to be considered as a group of principal bronchi. Phylogenetically they are one of the most sharply differentiated groups of the stem. We have designated the dorsal series, D. 2, D. 3, D. 4, etc., to keep their numerals in harmony with that of the larger lateral bronchi, although it is clear, of course, that our D. 2 is the first element of the dorsal series.

VENTRAL BRONCHI.

Because of their extreme variability, Aeby looked upon this group as accessory bronchi, which had their origin in the lateral series and subsequently wandered to take up a position on the stem bronchus. Among this group he classifies the Bronchus cardiacus. These conclusions were obtained from the study of adult specimens, so Aeby brings no definite proof of their wandering. His does not mention them, while Willach, also without evidence, seems to accept Aeby's view. They are, according to Robinson, a definite group of independent bronchi, which he terms ventral. Narath accepts the older view of Aeby, but like that author, his conclusions, with the exception of the infracardiac bronchus, are drawn from comparative study of corrosions of the adult lungs. Moreover, even in the case of the Bronchus cardiacus, Narath acknowledges embryology brings no direct proof of a wandering in the sense of Aeby. d'Hardiviller clings to the expression accessory, although he regards this group, which he terms anterior bronchi as independent derivations of the stem bronchus. In the latter view he is supported by Nicholas and Dimitrova who, like Robinson, term them ventral branches of the stem. The results obtained from the pig indicate that the ventral bronchi are independent derivatives of the stem and do not form first on the lateral series and then secondarily become transplanted on to the main bronchus.

VENTRAL ?, BRONCHUS CARDIACUS.

This bronchus Aeby looked upon as the most important of the ventro-accessory group. Derived primarily from the second lateral bronchus, it takes its place upon the stem bronchus between it and L. 3. In many species it supplies a separate lobe, the Lobus infracardiacus instead of

being included in the Lobus inferior. In his investigations on the human lung, His, from its size, the position of its origin, and its precocious development looks upon the Bronchus cardiacus as an independent element which appears out of the regular schematic order, a view with which Willach agrees. Robinson, while accepting the ontogenetic interpretation of His, believes with Aeby in its phylogenetic derivation from the Lateral 2. In holding that it may arise either from the second lateral or the stem bronchus, Zumstein takes a combined view, that is to say, in some instances it is an accessory bronchus and in others it is an independent structure. Narath is a most decided supporter of Aeby's doctrine, both from an embryological and a comparative point of view, but thinks L. 3 and L. 4, as well as the second lateral bronchus may give rise to this trunk, a view in which he is supported by Merkel and Blisnianskaja. d'Hardiviller and Nicholas and Dimitrova, however, look upon it as one of the principal branches of the stem bronchus. In the pig, the independence of this element is shown with great clearness where it forms the largest element of the ventral group of bronchi. Its hyperdevelopment apparently results from the increase in the respiratory surface by the utilization of the space between the heart and liver medialwards to the two stem bronchi for lung tissue. It is unpaired, like Lateral 1 and with that element destroys the symmetry of the tree.

MEDIAL BRONCHI.

Aeby's idea in classifying this group as dorsoaccessory, that is to say, branches originating on the dorsal bronchi and wandering on to the stem bronchus was practically the same as in the case of his ventroaccessory group, namely, their inconstancy and the existence, in a series of adult lungs, of bronchi, which looked like transition stages between the origin of a medial element on a dorsal trunk and its final position on the stem bronchus. Willach, without definite observations, supported this view, while Robinson, who calls them dorsointernal bronchi and believes them accessory, in the sense of Aeby, describes their origin by means of a splitting of the division between the two buds of a dorsal bronchus down to the main bronchus leaving the inner one of the buds with an independent origin on the stem. Zumstein speaks of them as medial and independent in which he has the support of Nicholas and Dimitrova and d'Hardiviller, although the latter designates the group as an internal series. Merkel accepts the older doctrine of Aeby. Narath, also, believes from both embryological grounds and from com-

parative anatomy that these bronchi can be traced as branches of the dorsal group. A criticism of his view has already been given. In the pig, they are irregular, but independent products of the stem. As they never occur more than a short distance above L. 4, we find the reason lies in the presence of the œsophagus, which prevents the development of medial bronchi above that level.

The main results of the preceding paper may be expressed in the following:

Résumé.

1. The anlage of the lungs in the pig is unpaired and asymmetrical. It arises from the ventral part of the head gut behind the Sinus venosus, as a ventral outgrowth, preceded by a lateral flattening of the foregut below the gill pouches and the appearance of longitudinal furrows, which divide the fore gut into two parts, a ventral respiratory portion and a dorsal digestive segment. From the lower part of the anlage the lungs arise, from the upper the trachea. If there is a serial phylogenetic association between the pulmonary anlage and the gill pouches, as some authors maintain, the connection is lost in the pig, for the lungs originate well below the gill area and distinctly ventralwards to the series of bronchial pouches. From the caudal extremity of the pulmonary anlage, arise two lateral outgrowths, giving rise to the stem bronchi. These, like the anlage itself, are asymmetrical, the right growing lateralwards and caudalwards, while the left extends almost directly horizontal. Then the respiratory and digestive portions begin to separate, a process, which begins from the caudal end of the anlage and extends upwards along the line formed by the two longitudinal furrows, freeing the respiratory apparatus from the œsophagus. In its subsequent growth, the pulmonary anlage enlarges, the tips of the stem bronchi dilate, and begin to bend dorsalwards around the œsophagus. This results in the formation of the primitive lung sacs. At this time, the production of the bronchi begins. They are readily divided into four series from the topography of their origin, namely, lateral, dorsal, ventral, and medial.

2. The first lateral bronchus, the so-called "eparterial bronchus," is, in the pig, unpaired and arises as a lateral outgrowth from the right side of the trachea, just above the roots of the two stem bronchi. It is distinctly lateral in origin and bears a serial relationship to the remainder of the lateral bronchi. Its position in mammals varies, sometimes it is on the stem bronchus, but it is often situated on the trachea.

This difference can usually be explained by the point of origin of the two stem bronchi with reference to the pair designated as Lateral 2. If the stems originate low down, then Lateral 1 is thrown on to the trachea, while if their origin is higher up, the first lateral arises from the stem bronchus. Apparently Lateral 1 is characteristic of mammals and, according to Aeby, of birds. A bronchus corresponding to it has not been found either in reptilia or amphibia. In almost all mammals it is an unpaired element. No satisfactory proof has even been brought to show a bilateral development of Lateral 1 with a subsequent degeneration of the left bronchus, notwithstanding the fact that this process has been described in two species. At no time in the life history of the pig is there a Lateral 1 formed on the left side. There is furthermore no embryological evidence to show a relationship between Lateral 1 and the dorsal series of bronchi. These characteristics are secondary and result from the antagonistic effects of the growth of Lateral 1 and Lateral 2. The latter is forced somewhat ventralwards, while the former is pressed dorsalwards, until its lower branches lie above the dorsal series of bronchi.

3. The remainder of the lateral series originate in succession from the lateral side of the stem bronchus as lateral outgrowths or hernia-like expansions of the wall of the stem bronchus near the terminal bud. These elements in their growth outwards finally reach the chest wall. Here they are compelled to grow in the space between the ribs and the liver and consequently follow the curvature of the chest wall which ultimately gives them, more or less, the appearance of ventral bronchi, a fact which led Aeby, who studied only the finished tree, to call them the ventral series.

4. The dorsal series of bronchi, originating like the lateral group as outgrowths from the stem bronchus, are usually paired. They alternate with the paired lateral bronchi and are independent productions of the stem. They do not either ontogenetically or phylogenetically originate from the lateral bronchi. For convenience, the first pair are called Dorsal 2, to keep the designation harmonious with the larger series of lateral bronchi.

5. The ventral bronchi originate as outgrowths from the ventral surface of the stem. They, like the other series, are independent productions of the main bronchus. They are not originally formed on the lateral bronchi and subsequently transferred to the stem bronchus. Consequently, they are chief bronchi and not accessory in the sense of Aeby. In the pig and in the great majority of mammals, left Ventral 2 is

suppressed. With the absence of left Lateral 1, it destroys the absolute symmetry of the mammalian lung. The cause for the remarkable hyperdevelopment of the Ventral 2 on the right side in most mammals is undoubtedly due to the effort to increase the respiratory area by filling the space that intervenes between the heart and diaphragm with the Lobus infracardiacus. The remainder of the ventral series are usually paired in the pig and like the dorsal series ordinarily alternate with the larger lateral bronchi. As a rule their roots are placed on the ventral surface of the stem midway between the adjacent lateral elements and opposite the corresponding dorsal bronchi. The first ventral element is designated Ventral 2 on account of its topographical relationship to Lateral 2.

6. The medial bronchi are, like the other series, produced by medial outgrowths from the stem. They are not formed on the dorsal bronchi and then transferred to the stem. They rarely occur higher than the level of Lateral 4 and are extremely irregular in their arrangement.

7. Noteworthy are the great variations found in the production of the various bronchi. The lateral series are by far the most constant elements of the tree. Still, it is not uncommon to find either an extra element formed or else to see one of the usual elements suppressed. As the common number of lateral elements is six on the right side and five on the left, the extremes may vary between five and seven on the right and four and six on the left. In the case of the dorsal series, the variation is even more marked than in the lateral, thus, one element may be suppressed, leaving the dorsal area between two adjacent lateral bronchi naked or, else, an extra element may be formed, giving two dorsal elements in a single interspace. The ventral series is still more variable than the dorsal, so much so, in fact, as to make it uncommon even in the pig where these elements are unusually well developed, to find a series complete, of course, with the exception of left Ventral 2, which is always suppressed. It is not uncommon to find several elements of this series absent at once. Like the dorsal bronchi, they may also be reduplicated in a single interspace. The medial bronchi are the most variable of the four types. They may not be present at all, they may be present only on one side, or they may be reduplicated in a single interspace, but, in the pig, they never occur higher on the stem than the level of the fourth lateral bronchus. The reason for this fact lies in the presence of the œsophagus above this point, which allows no space for the development of medial elements from this portion of the stem bronchus.

8. The following formula would represent the complete series of principal bronchi in the lung of the pig:

TRACHEA.

Lateral 1.	
Right Stem Bronchus.	Left Stem Bronchus.
Lateral 2.	Lateral 2.
Dorsal 2.	Dorsal 2.
Ventral 2.	
Lateral 3.	Lateral 3.
Dorsal 3.	Dorsal 3.
Ventral 3.	Ventral 3.
Lateral 4.	Lateral 4.
Dorsal 4.	Dorsal 4.
Ventral 4.	Ventral 4.
Medial 4.	Medial 4.
Lateral 5.	Lateral 5.
Dorsal 5.	Dorsal 5.
Ventral 5.	Ventral 5.
Medial 5.	Medial 5.
Lateral 6.	Lateral 6.

It is extremely rare to find a tree as complete as the one expressed in this formula. A number of bronchi may be missing or else some may be reduplicated.

9. The whole series of bronchi show a most remarkable adaptation to the space in which they have to grow. This is true of both the chief bronchi as well as their smaller subdivisions. When, for example, a bronchus is suppressed, an adjacent branch will grow into the area usually supplied by the missing element, substituting for its loss. It is in this way that we obtain the large series of pictures which suggest a wandering of the secondary branches from the lateral and dorsal elements on to the stem bronchus. After a careful study of this point, it may be definitely stated that bronchi never wander. They remain firmly fixed on the stem or side branches where they originate. Not uncommonly their direction may be altered, however, by changes in the space in which they develop.

This response on the part of the growing bronchi to their space relationships is also shown in the course or direction of the principal elements as well as their secondary branches. We have, therefore, Lateral 1 produced and growing into the area between the upper part of the heart and chest wall. Owing to the larger space just beside the vertebral column and the antagonism between it and Lateral 2, the lower branches of Lateral 1 are forced dorsalwards until it resembles superficially a

dorsal bronchus. The second lateral bronchi develop in the region between the chest wall, heart, and liver. The area in which the remainder of stem has to grow has in cross-section practically the shape of an isosceles triangle. The stem, occupying a point about the middle of the base, sends three sets of branches, namely, dorsal, lateral, and ventral, directed into the angles of the triangle where they would have the most freedom to develop. Between the roots of the two stem bronchi runs the œsophagus, leaving no place for the development of median branches in this region. At the level of Lateral 4, however, below the œsophagus more room occurs and, consequently, we observe in this region the formation of medial bronchi. Undoubtedly the difference in the branching of the stem in the Lobus inferior of the human lung when compared with the pig may be sought in its altered topography owing to the erect posture which changes principally the position of the liver.

This adaptation on the part of the lungs to their environment is to be expected for they are relatively late accessions to the animal economy and are of no known use to the organism during the period of gestation. Accordingly as the heart and liver are both phylogenetically older than the lungs and also are of known functional value during fœtal life, it is natural that the latter should adapt themselves to the early needs of older organs.

10. The growth of the main series of bronchi is monopodial in character, that is to say, they are produced without a definite division of the end bud. New elements are not always produced from the end bud, but may be formed from the stem some distance from its terminus. The process is successive, that is to say, the elements are produced one after another from above downwards, recapitulating the method of growth shown in simpler animals like the reptiles, for example. When a new element is about to be produced, one notes an increase in the number of karyokinetic figures in the epithelium in the region of the new branch. The basement membrane becomes less distinct and the connective-tissue nuclei in the surrounding mesoderm are more closely packed together. In this region a slight bulging of the epithelium is then noted, which increases until a small elevation is raised upon the surface of the stem. This increases in size, yielding a rounded projection, which gradually emancipates itself and gives rise to a new bronchus. The process is essentially the same whether it occurs in the neighborhood of the terminal bud or higher up on the stem. In general, we may say, the lateral and medial elements are produced nearer the terminal end of the main

bronchus, while the dorsal and ventral elements are formed somewhat higher up, often where the stem has regained its cylindrical form.

Subsequent division of the branches may occur either by monopody or dichotomy. Often monopodial production of buds persists for one or two generations on the main bronchi, then the method becomes dichotomous, either equal or unequal in nature depending somewhat on the space in which the bronchi have to divide. In the case of equal division of the bud, however, one fork grows on to become the stem while the other remains as the side branch. The first division of the main bronchi may, it is well to note, be dichotomous as in the case of Lateral 1 and Lateral 2. Thus in its growth, the mammalian lung recapitulates the history of the simpler lungs of lower animals.

11. The pulmonary arteries in the pig arise from the pulmonary arches as Bremer has described. At first, they run parallel, then bend towards each other, sending out anastamoses, which yield finally a common trunk with two origins above and two arteries below. Later the upper part of the right artery degenerates and with it the right pulmonary arch. At 5 mm. before the pulmonary arteries may be followed as far as the anlage of the lungs, the pulmonary vein may be seen as a slight ingrowth from the undivided portion of the Sinus venosus, passing through the Mesocardium posterior towards the pulmonary anlage. It forms almost in the medial plane. With this establishment of the venous outlet ventralwards to the anlage, the arteries, as the growth of the organ proceeds, are naturally developed from the capillary plexus on the dorsal side of the primitive bronchi. This fixes the arteries with reference to the stem bronchi before any of the side branches are produced. As the pulmonary anlage projects some distance ventralwards from the head gut, Lateral 1, the "eparterial" bronchus, develops above the artery, while Lateral 2 and the remainder of the principal branches originate below. Thus, the two regions of the tree have a different topography with reference to the pulmonary artery, but this vessel has no fundamental influence on the structure of the two parts, nor does it differentiate the tree into two regions of different morphological significance as Aeby has maintained.

The entire primitive tree is surrounded by a capillary plexus. As the bronchi grow, and produce new branches, arteries are developed from this plexus on the dorsal side of the tree as the artery lies dorsalwards and lateralwards to the stem. From this position, arteries to the lateral bronchi run out above and behind them. The branches to the dorsal bronchi pass dorsalwards along the lateral aspect of these elements. To

the ventral series, arteries pass around the lateral aspect of the stem bronchus beneath the root of the corresponding lateral bronchus to gain the outer aspect of the ventral bronchus along which they run. The medial bronchi receive their supply from branches that originate from the main artery and pass around the dorsal aspect of the stem to run on the dorsal surface of the medial bronchi. As the right pulmonary artery runs ventralwards to Lateral 1 the artery to that bronchus develops on its ventral surface.

In the younger stages, both the aortic arch and the Ductus arteriosus lie well above the level of Lateral 1. As the embryo increases in age, there is a gradual descent of the heart and with it, the great vessels. At 15 cm. one observes the Ductus arteriosus at the level of Lateral 1; at 22 cm. the aortic arch reaches this point, while at birth both vessels lie below the bronchus.

12. The pulmonary vein develops in pigs about 5 mm. long as an ingrowth from the undivided portion of the Sinus venosus at the level of the pulmonary anlage. As the stem bronchi increase in size, right and left pulmonary veins develop from the capillary plexus which surround them. These, naturally, form on the ventral surface, with the bronchi between them and the arteries. Similarly, as the various principal bronchi are produced from the stem bronchus, veins are formed from the capillary plexus. The veins from the lateral bronchi lie below and ventralwards to the bronchi, those from the dorsal elements run along the medial aspect of the air passages to empty into pulmonary veins lying ventralwards to the stems. The veins from the ventral bronchi extend along the medial aspect of the bronchus and terminate directly into the pulmonary veins; those from the medial bronchi extend along their ventral surface to empty in the larger veins accompanying the stems. The vein from Lateral 1 runs along the ventral aspect of the bronchus somewhat ventralwards to the corresponding artery. This forms the single exception to the general alternation of artery, bronchus, and vein. As the embryo increases in age, the Vena pulmonalis, which originates near the midline, is gradually pushed to the left by the increasing asymmetry of the heart, until it finally comes to lie over the area of the stem bronchus where a left Ventral 2 would have developed if such a bronchus were present. The hyperdevelopment of the Bronchus infracardiacus associated with the development of the Vena cava inferior to the right of that bronchus aids in pushing the Vena pulmonalis to the left.

13. The asymmetry of the mammalian lung is associated with the

asymmetrical development of the heart and its great vessels. In the descent of the aortic arch and the Ductus arteriosus during embryonic life from a point above the origin of Lateral 1 to a point below, we have an explanation for the suppression of this element on the left side, for if this bronchus were formed, both aorta and the Botallian duct would be caught upon it and their descent prevented. Likewise the Vena pulmonalis appears in the midline and is carried to the left until it finally rests on the portion of the stem where a left Ventral 2 should develop. The usual suppression of these two elements, therefore, must be looked upon as a phylogenetic provision to allow for the descent of the great vessels on the one hand and the shifting of the Vena pulmonalis on the other. It is noteworthy that in those animals where these bronchi are formed on both sides, they are so situated as to offer no resistance to either of these features of the development of the great vessels.

14. The mesodermic portion of the lungs is derived from the general mesoderm about the head gut. As the bronchi appear, this is pushed out into the primitive cœlom to form two irregular swellings, marking the anlagen of the two wings of the lungs. With the appearance of Lateral 1, on the right side, and Lateral 2, on each stem bronchus, swellings are observed on the two simple lungs just over these bronchi, giving rise to the simplest forms of the Lobus superior, Lobus medius, on the right side, and the Lobus superior on the left. The remainder of the mesoderm about the stem bronchus forms the anlage of the Lobus inferior on each side. With the formation of Ventral 2, the Bronchus infracardiacus, a swelling from the mesoderm forms over it which is the anlage of the Lobus infracardiacus. These swellings are first surrounded by shallow grooves, which with the rapid growth of the bronchi beneath, rapidly develop into deep fissures separating the various lobes from each other. With the further growth of these bronchi and the appearance of the series of bronchi on the stem, projections and fissures are formed over and between them and in the mesoderm. These are equivalent in all respects except in age and size, to the earlier fissures and swellings, but, under ordinary circumstances, never give rise to distinct lobes. This is due to the more rapid growth of the first bronchi, to the gradual increasing density of the mesoderm, and, lastly, to the environment of the several lobes of the lung. The right Lobus superior, containing Lateral 1 does not belong to the dorsal region of the lung as some authors hold, but to the lateral. The characters which make it appear as a dorsal segment are secondary and not primary. Likewise the portion of the left Lobus superior containing the apical bronchus belongs to the lateral

region and not to the dorsal. As in the case of the right Lobus superior, its dorsal characteristics are secondary. This segment is to be compared to the portion of the right Lobus medius which contains the main dorsoinferior bronchus. Moreover, the entire left Lobus superior is the ontogenetic equivalent of the right Lobus medius. The right Lobus superior is an unpaired lobe and has no equivalent in the left lung. The same thing is true of the Lobus infracardiacus.

Lobe formation varies greatly in different species. In the majority of mammals, there are three or four lobes on the right side, arising from Lateral 1, Lateral 2, Ventral 2, and the stem bronchus, while, on the left side, there are ordinarily two formed from Lateral 2, and the stem. Extremes of variation occur, however, between a lobeless lung in which none of the bronchi subdivide it and a multilobar lung in which most of the principal bronchi have segmented the wing into a series of small lobes. Apparently, the division of the lung into lobes is of no general morphological significance.

15. In the light of recent researches on the reptilian, amphibian, and avian lung, it is possible to take a new viewpoint for the development of the mammalian lung. The lungs of lower animals, we now know, are products of monopodial growth. The simple lungs of reptilia are capable of producing monopodially outgrowths in any direction (Hesser). These may become specialized in certain species and have a definite topography. As we mount the animal scale, the necessity of an increased respiratory surface finally results in the transformation of the original simple lung into a conducting apparatus, which is represented in the mammalian lung by the stem bronchus and its chief branches. The simple lungs may no longer be compared to the Lobuli respiratorii of the mammalian lung, for the latter represent new elements which with the increased respiratory surface are added peripherally to the simpler lungs as these become transformed into bronchi. With the addition of these new elements, the respiratory function also wanders peripheralwards, so that the portion of the mammalian tree which represents the simpler lungs undergoes a change of physiological function. Its phylogenetic relationship to the simple lungs is shown by the monopodial growth of the mammalian stem bronchus and its principal branches, which recapitulate ontogenetically the growth process of the simple lungs before producing dichotomously the prepheral respiratory structures which are used in mammalian respiration. In certain animals, moreover, the stem bronchus and its branches retain for a period in their life history their respiratory function. In monotremes and marsupialia,

the young are transferred to the pouch and compelled to carry on their own respiration when only the stem bronchus and its chief branches are formed. The ordinary respiratory structures used in the adult stage, are produced at a later period. We have, thus, both a physiological and an ontogenetic proof that the simple lungs correspond, in mammals, only to the stem bronchus and its chief branches.

The great majority of mammalian lungs are asymmetrical, the asymmetry consisting in the presence of an unpaired Lateral 1 and an unpaired Ventral 2, both of which occur on the right side. Some mammalian lungs are symmetrical and considerable effort has been made to explain all the asymmetrical lungs on the basis of the minority of symmetrical ones. The asymmetrical lung, however, must be regarded as typical for mammals. The two bronchi responsible for the asymmetry are, so far as we know, characteristic of the mammalian and avian (Aeby) lung as similar bronchi have never been described in the lungs of lower animals. The cause for the asymmetry, apparently lies in the necessity of leaving space for the descent of the heart and great vessels, by the suppression of left Lateral 1, on the one hand, and to allow room for the shifting of the heart which draws the Vena pulmonalis to the left by the suppression of left Ventral 2, on the other. In those lungs where these two elements, which are usually missing, are found, they are apparently so placed as not to interfere with these features of the development of the heart.

16. In the organogenesis of the lungs, we have the stem and main bronchi consisting of simple tubes lined by a double layer of epithelium, the inner of which is columnar, while the outer is composed of smaller polygonal cells. This simple tube is surrounded by a membrana propria produced largely by the deposit of fibrils from the exoplasm of the connective-tissue syncytium, composing the mesoblastic portion of the lungs at this early stage. As the bronchi grow, a layer of spindle cells differentiate from the mesoderm, which are transformed into the muscular coat of the bronchi. Later still, a chondrification of the perimuscular syncytium takes place from which the cartilaginous rings of the trachea and the bronchial cartilages are formed. With these changes, the connective-tissue fibrils become grouped into trabeculæ about the bronchi and in the submucosa. Later, the mucosa is thrown into a series of longitudinal folds, while from the cuticular border of the inner row of cells, cilia develop. From the bottom of the crypt-like invaginations formed by the longitudinal folds of epithelium, glands begin to grow into the submucosa, which sometimes pass between the developing muscle

bundles into the deeper layers of this coat. As this process takes place, there is a differentiation of some of the epithelium into goblet cells, a process, which one also observes in the glands, giving rise to a series of submucous glands with partly serous and partly mucous cells. While these changes occur in the mucosa, the cartilages are also growing, and with them a further differentiation of the framework into distinct fibrous trabeculæ takes place. As we follow the bronchi peripheralwards, they become simpler and essentially younger in structure and yet, develop their adult characteristics in precisely the same way. The epithelium soon becomes single layered and of a columnar type as the periphery is reached. Finally it takes on a distinct, flat, cubical form. The Lobuli respiratorii begin to develop in pigs about 19 cm. long by a slight dilatation of the growing ends of the bronchi. These represent the bronchioli. Later Bronchioli respiratorii are then formed, having a progressively flattened epithelium, which runs over into Ductuli alveolares. These are present at the age represented by a pig 22 cm. long. Subsequently, Atria, Sacculi alveolares, and Alveoli pulmonis form in the prenatal period, all of which have the characteristic flattened respiratory epithelium. And finally, after birth, there is a dilatation of the lobules and a further flattening of the epithelium occurs, and before the pig is half grown, a muscle layer develops about the air passages as far as the Atria, where it stops in sphincter-like bands. One finds at no period in the life history of the pig's lung, openings or fenestræ which communicate between adjacent respiratory lobules. The latter form independently at the growing ends of the tree and as they approximate each other, the interalveolar framework can always be demonstrated between them without interruptions suggestive of fenestræ connecting adjacent alveoli.

17. The framework of the lungs develops from a general syncytium forming the mesodermic anlagen of the lung wings. By a gradual differentiation of connective-tissue fibrils from the exoplasmic part of the syncytium, the framework becomes denser and, finally, at 8 cm., a suggestion of lobulation is obtained about the end branches of the growing bronchi. Within these connective-tissue lobules, the framework differentiates as the embryo grows, forming simultaneously basement membranes for the young bronchial buds. At the same time, the interlobular fibers and those below the pleura, unite to form trabeculæ. As the Lobuli respiratorii, towards the end of fœtal life, begin to impinge on each other, the interalveolar framework and the two adjacent basement membranes are pressed together into a single wall or septum in which the

blood-vessels run. These lobules persist until adult life, although they may become compound by the rupture of the interlobular septa and the subsequent confluence of several adjacent lobules. This process ordinarily takes place at the base, leaving the periphery of the compound lobule separated by partial septa.

18. The lymphatics appear at the root of the lung in an embryo 4-5 cm. in length. Accompanying the bronchi and pulmonary vessels, they gradually grow in for some distance until the smaller air passages are reached, when they leave these structures and grow towards the pleura in the interspaces between the smaller bronchi, in what represent the primitive interlobular spaces. In this way they aid in the differentiation of the connective-tissue lobules. The reason for this course is not entirely clear, but it may be due to the increasing density of the framework about the bronchi, which forces the later-appearing lymphatics into the interlobular spaces as a locus minoris resistentiæ. Upon reaching the pleura, they turn and form a plexus in the subpleural connective tissue. Here and there, they may be seen penetrating the lobules, but cannot be followed for any distance in them. At 23 cm., the first evidence of the submucous plexus is seen in the stem bronchi.

LITERATURE.

Aeby.—Der Bronchialbaum der Saügethiere und des Menschen. Leipzig, 1880.

v. Baer.—Ueber Entwickelungsgeschichte der Thiere. Königsberg, 1828.

Bichat.—Anatomie descriptive. Paris, 1829.

Blisnianskaja.—Die Entwickelungsgeschichte der menschlichen Lungen. Diss. Zurich, 1904.

Bonnet.—Handbuch d. vergl. Histologie u. Physiologie der Haussäugethiere. Bd. 2, Berlin, 1892.

Bremer.—American Journal of Anatomy. Vol. I, 1902.

——— American Journal of Anatomy. Vol. III, 1904.

Cadiat.—Jour. de l'Anatomie et de la Physiologie. 1877.

Councilman.—Journal Boston Med. Soc. Vol. IV, 1901.

Ewart.—The Bronchi and Pulmonary Blood-vessels. London, 1899.

Fischelis.—Beitrage zur Kenntniss der Entwicklungsgeschichte der Lunge. Diss. Berlin, 1885.

Flint.—Johns Hopkins Hospital Reports. Vol. X, 1900.

——— Journal of Medical Research. Vol. VII, 1902.

——— (1) American Journal of Anatomy. Vol. II, 1903.

——— (2) Archiv. f. Anat. u. Ent. Anat. Abth. 1903.

——— (1) Anatomischer Anzeiger. 1906.

——— (2) Anatomischer Anzeiger. 1906.

Fol.—Recueil zoologique suisse. T. 1, 1884.

GEGENBAUER.—Grundriss der Vergleichenden Anatomie. Leipzig, 1874.
——— Vergleichende Anatomie der Wirbelthiere. Bd. 2, 1901.
GÖTTE.—Beitrage zur Entwicklungsgeschichte des Darmkanals im Hünchen. Tübingen, 1867.
——— Die Entwicklungsgeschichte der Unke. Leipzig, 1875.
——— Zoölogischer Jahrbücher Anat. Abth. 1904.
GREIL.—Anat. Hefte. Bd. 29, Hft. 3, 1905.
GUIEYSSE.—Jour. de l'Anatomie et de la Physiologie. T. 34, 1898.
D'HARDIVILLER.—(1) Comptes Rendus de la Société de Biologie. Vol. III, 1896.
——— (2) Bibliographie Anatomique. Vol. IV, No. 5; Vol. V, No. 1, 1896-1897.
——— (1) Bibliographie Anatomique. Vol. V, No. 1, 1897.
——— (2) Bibliographie Anatomique. Vol. V, No. 6, 1897.
——— (3) Comptes Rendus de la Société de Biologie. Vol. 4, November 20, December 4, December 11, 1897.
HESSER.—Anat. Hefte. Bd. 29, Hft. 2, 1905.
HIS.—Untersuchungen über die erste Anlage des Wirbelthierleibes. Leipzig, 1868.
——— Arch. f. Anat. u. Entwick. Anat. Abth. 1887.
HOCHSTETTER.—Zeit. fur wissenschaft. Mikroskopie. Bd. 15, 1898.
HUNTINGTON.—Annals of the New York Academy of Science. Vol. XI, 1898.
JUSTESEN.—Arch. f. Mik. Anat. 1900.
KASTSCHENKO.—Arch. f. Anat. u. Phys. Anat. Abth. 1887.
KÖLLIKER.—Entwicklungsgeschichte des Menschen. Leipzig, 1879.
KÜTTNER.—Virchow's Archiv. 1876.
LEYDIG.—Lehrbuch der Histologie des Menschen und der Thiere. Frankfort A/M, 1857.
LAGUESSE ET D'HARDIVILLER.—Comptes Rend. Soc. Biol. T. 5, 1898.
MALL.—American Journal of Anatomy. Vol. I, 1902.
MERKEL.—Handbuch der Anatomie des Menschen (von Bardeleben). Jena, 1902.
MEYER.—Lehrbuch der Anatomie des Menschen. Leipzig, 1861.
MILLER.—Journal of Morphology. 1893.
——— Arch. f. Anat. u. Phys. Anat. Abth. 1900.
MINOT.—Human Embryology. New York, 1892.
MOSER.—Arch. f. Mik. Anat. Bd. 60, 1902.
MÜLLER.—Jenaische Zeitschrift fur Naturwissenshaft. Bd. 32 (N. F. Bd. 25), 1898.
NARATH.—Verhandl. der Anat. Gesellsch. 1892.
——— Zoologische Forschungsreisen in Australien u. dem Malayischen Archipel. (Semon), Jena, 1896.
——— Bibliotheca medica. Abth. A, Hft. 3, 1901.
NICHOLAS AND DIMITROVA.—Comptes Rendus de la Société de Biologie. 1897.
RATHKE.—Verh. d. Kais. Leop. Carol. Akad. d. Nat. Bonn, 1828.
——— Entwicklungsgeschichte der Natter. Königsberg, 1839.
REMAK.—Untersuchungen über die Entwickelung der Wirbelthiere. Berlin, 1855.
ROBINSON.—Journal of Anatomy and Physiology. 1889.

Rüdinger.—Topographisch-chirurgische Anatomie des Menschen. Stuttgart, 1873.
Sakurai.—Anatomischer Anzeiger. 1904.
Schmalhausen.—Anatomischer Anzeiger. Bd. 27, 1905.
Schmidt.—Cited by His (87). 1870.
Seessel.—Arch. f. Anat. u. Phys. Anat. Abth. 1877.
Selenka.—Studien uber die Entwickelungsgeschichte der Thiere. Heft 1, das Opossum. Wiesbaden, 1887.
——— Zeit. f. wiss. Zool. Bd. 16, 1866.
Stieda.—Zeit. f. wiss. Zool. Suppl. Bd. 1878.
Stoss.—Untersuchungen über die Entwicklung der Verdauungsorgane. Diss. Leipzig, 1892.
Thoma.—Untersuchungen über die Histogenese und Histodynamik des Gefässsystems. Stuttgart, 1893.
Uskow.—Arch. f. Mik. Anat. Bd. 22, 1883.
Weber and Buvignier.—Bibliographie Anatomique. T. 12, 1903.
Willach.—Beitrage zur Entwicklung der Lunge bei Saügethieren. Osterwieck-Harz (Vickfeld), 1888.
Zumstein.—Sitzungsberichte der Gesell. zur Beförd. d. gesammt. Naturwiss. zu Marburg. Marz, 1889.
——— Sitzungsberichte der Gesell. zur Beförd. d. gesammt. Naturwiss. zu Marburg. Februar, 1891.
——— Sitzungsberichte der Gesell. zur Beförd. d. gesammt. Naturwiss. zu Marburg. Marz, 1892.
——— Sitzungsberichte der Gesell. zur Beförd. d. gesammt. Naturwiss. zu Marburg. Mai, 1900.

EXPLANATION OF THE PLATES.

Plate I.

Figs. 1-1½.

Figs. 1-20 are magnified 50 diameters. Pulmonary arteries red, pulmonary veins blue, bronchi white.

Fig. 1. Reconstruction of a portion of the head gut of a pig's embryo 3 mm. long. Ventral view.

Fig. 2. Dorsal view of the same reconstruction.

Fig. 3. Reconstruction of a portion of the head gut of a pig's embryo 5 mm. long. Ventral view.

Fig. 4. Dorsal view of the same reconstruction.

Fig. 5. Reconstruction of the bronchial tree of a pig 6 mm. long.

Fig. 6. Dorsal view of the same reconstruction.

Fig. 7. Reconstruction of the bronchial tree of a pig 7.5 mm. long.

Fig. 8. Dorsal view of the same reconstruction.

Fig. 9. Reconstruction of the bronchial tree of a pig 8.5 mm. long.

Fig. 10. Dorsal view of the same reconstruction.

FIG. 11. Reconstruction of the bronchial tree of a pig 10 mm. long.
FIG. 12. Dorsal view of the same reconstruction.
FIG. 13. Reconstruction of the bronchial tree of a pig 12 mm. long.
FIG. 14. Dorsal view of the same reconstruction.

PLATE II.

Figs. 15-19.

FIG. 15. Reconstruction of the bronchial tree of a pig 13.5 mm. long.
FIG. 16. Dorsal view of the same reconstruction.
FIG. 17. Reconstruction of the bronchial tree of a pig 15 mm. long.
FIG. 18. Dorsal view of the same reconstruction.
FIG. 19. Reconstruction of the bronchial tree of a pig 18.5 mm. long.

PLATE III.

Fig. 20.

FIG. 20. Dorsal view of the same reconstruction.

PLATE IV.

Figs. 21-25.

FIG. 21. Celluloid corrosion of the bronchial tree of a pig's embryo 5 cm. long. × 2.

FIG. 22. Celluloid corrosion of the bronchial tree of a pig's embryo 7 cm. long. × 2.

FIG. 23. Wood's metal corrosion of the bronchial tree of a pig's embryo 18 cm. long.

In this specimen one lateral bronchus on each side is suppressed, giving five laterals on the right and four on the left, instead of the usual complement of six and five respectively. Ventral 3 on both sides is suppressed. Substituting for these branches are ventral branches of the adjacent lateral bronchi, while on the right side a lateral division from the inferior branch of V. 2 also extends into the region usually supplied by right V. 3.

FIG. 24. Dorsal view of the same preparation.

Dorsal 3 on the left side is suppressed. It is compensated for partly by Dorsal 2 growing lower than usual and partly by branches from Medial 4 on that side. On the right side Dorsal 4 is reduplicated, the upper element growing dorsolateralwards, the lower directly dorsal. Medial 4 and 5 are present on both sides.

FIG. 25. Wood's metal corrosion of the lung of a suckling pig two days old. Ventral view. × 2.

Ventral 2 is broken off to show the dorsal bronchi. In places where the metal has passed into the smaller bronchi, the dichotomy is well shown. The branches are schematic in their arrangement with the exception of Ventral 5 on the left side, which is reduplicated, and an extra irregular lateral branch is interpolated on the right side.

ABBREVIATIONS.

b = Gill pouch.
a = Head gut.
c = Pulmonary anlage.
h = Ductus hepaticus.
o = Œsophagus.
ad = Arteria pulmonalis dextra.
T = Trachea.
d = Right stem bronchus.
s = Left stem bronchus.
as = Arteria pulmonalis sinistra.
v = Vena pulmonalis.
$L.$ 1, $L.$ 2, $L.$ 3, $L.$ 4, $L.$ 5, $L.$ 6, etc. = The lateral series of bronchi.
$D.$ 2, $D.$ 3, $D.$ 4, $D.$ 5, $D.$ 6, etc. = The dorsal series of bronchi.
$V.$ 2, $V.$ 3, $V.$ 4, $V.$ 5, $V.$ 6, etc. = The ventral series of bronchi.
$M.$ 4, $M.$ 5, etc. = The medial series of bronchi.
ap = Apical branch of left $L.$ 2.
m = Medial branch.
d = Dorsal branch.
l = Lateral branch.
v = Ventral branch.
s = Superior branch.
i = Inferior branch.

In the combined abbreviations:

di = Dorsoinferior branch.
li = Lateroinferior branch.
vs = Ventrosuperior branch, etc.

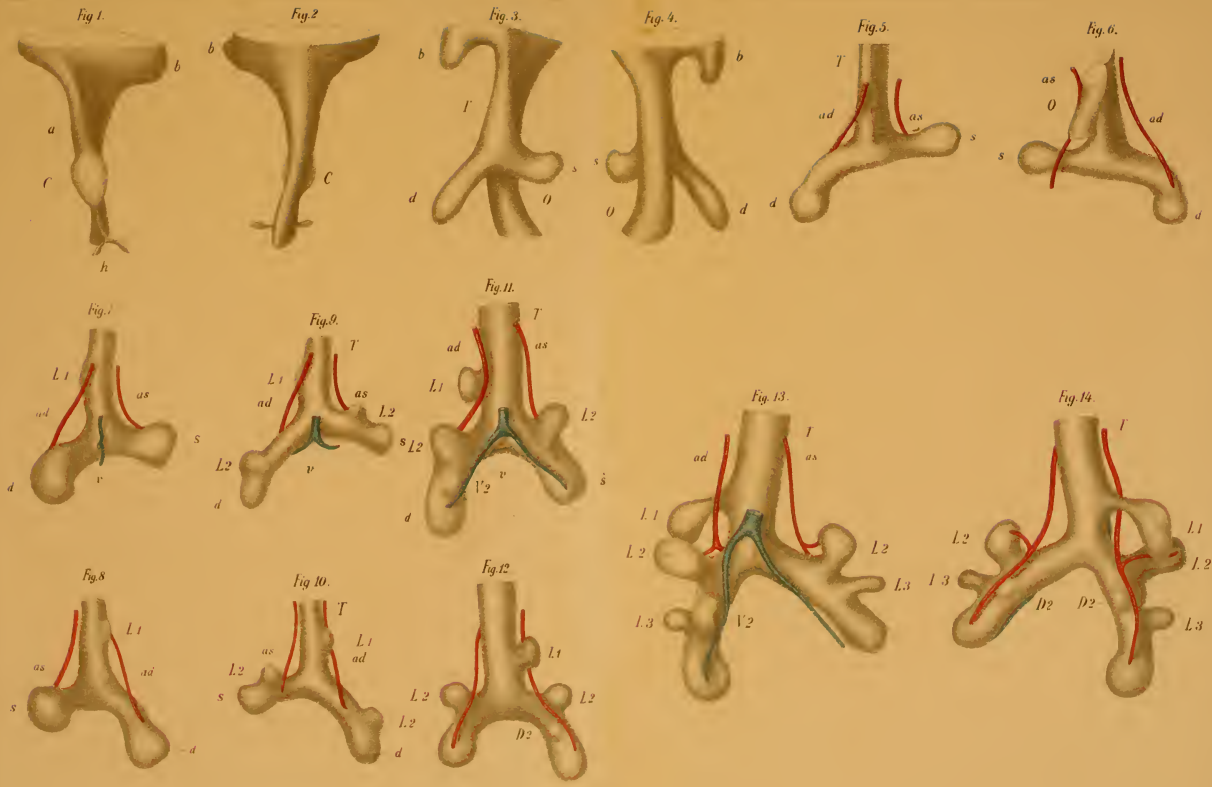
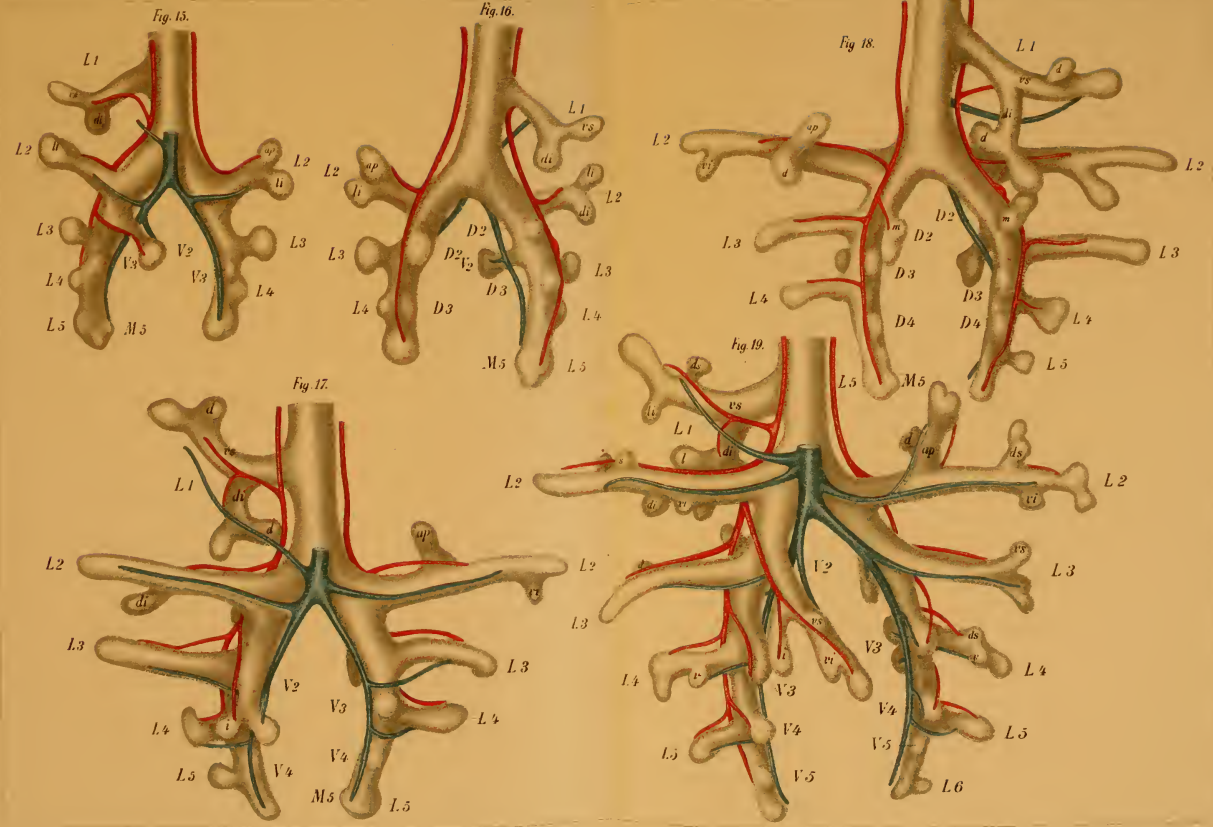


Fig. 1.
b
a
C
h
Fig. 2.
b
C
Fig. 3.
b
T
d
O
s
Fig. 4.
b
s
O
d
Fig. 5.
T
ad
as
s
d
Fig. 6.
as
O
ad
s
d
Fig. 7.
L 1
as
ad
s
d
v
Fig. 9.
T
L 1
as
ad
L 2
s
L 2
v
d
Fig. 11.
T
ad
as
L 1
L 2
s L 2
V 2
v
d
Fig. 13.
T
ad
as
L 1
L 2
L 2
L 3
L 3
V 2
Fig. 14.
T
L 2
L 1
L 3
L 2
D 2
D 2
L 3
Fig. 8
L 1
as
ad
s
d
Fig 10.
T
as
L 1
ad
L 2
s
Fig. 12.
L 1
L 2
L 2
L 2
D 2
d



Lith. Kunstanstalt Friedr Sperl Wien

Fig. 20.

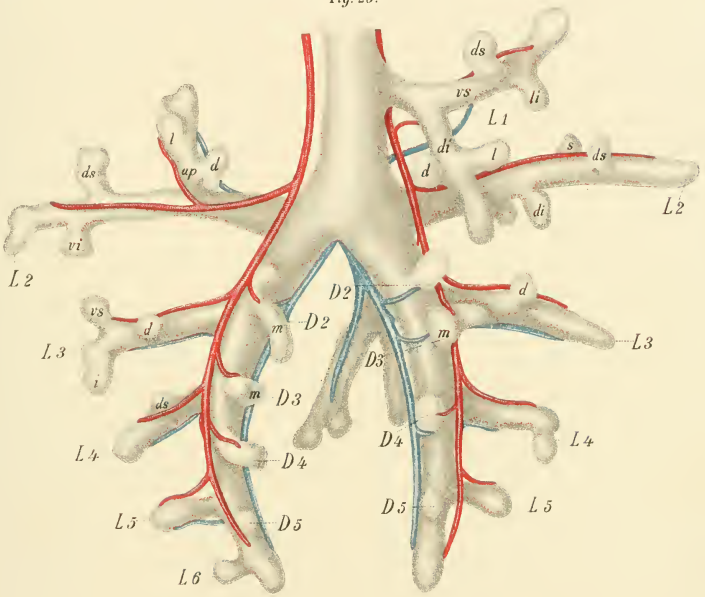

ds
vs
li
L 1
di
l
s
ds
d
d
di
L 2
l
ds
up
d
vi
L 2
D 2
d
vs
d
m
D 2
m
L 3
L 3
D 3
i
m
ds
D 4
D 4
L 4
L 4
D 5
L 5
L 5
D 5
L 6

Fig. 21.

Fig. 22.

Fig. 23.

Fig. 24.

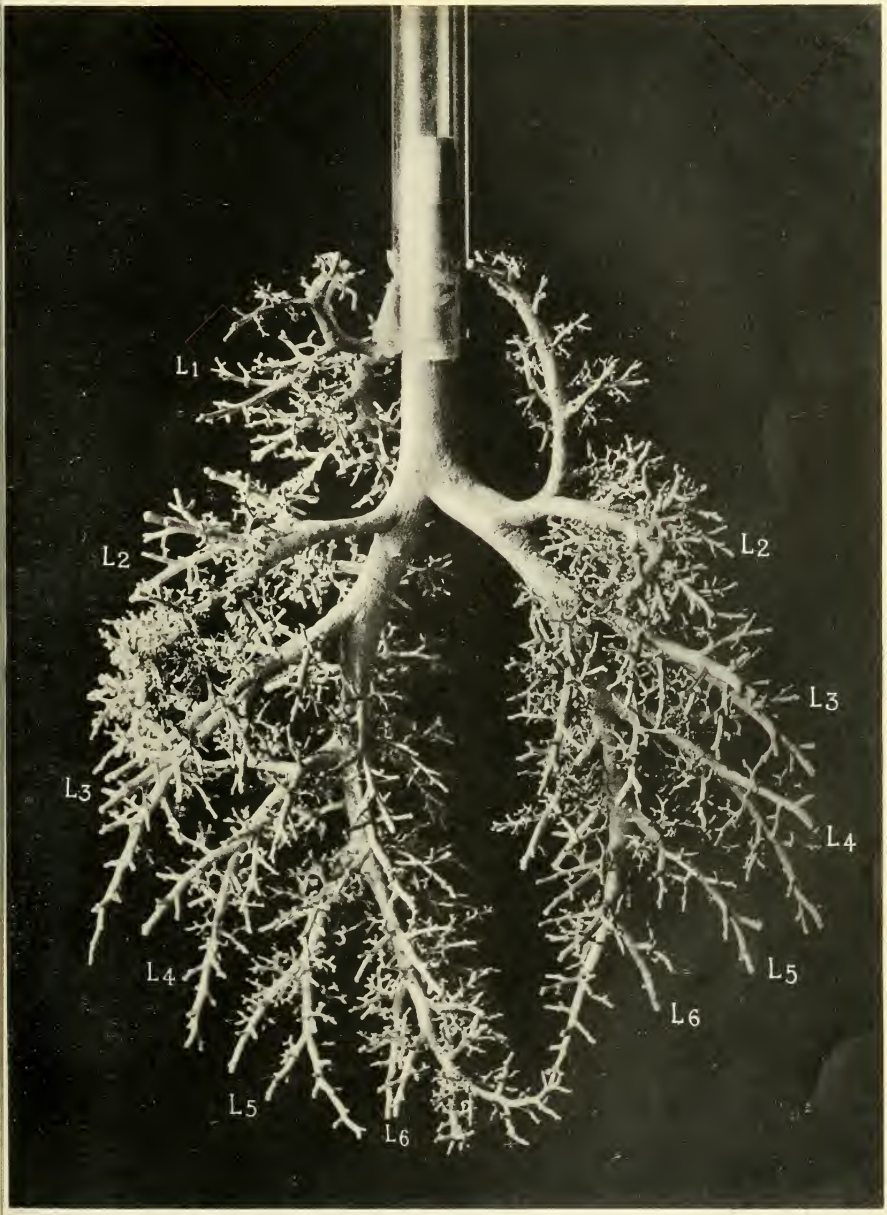


Fig. 25.

ON THE DEVELOPMENT OF THE MEMBRANOUS LABYRINTH AND THE ACOUSTIC AND FACIAL NERVES IN THE HUMAN EMBRYO.[1]

BY

GEORGE L. STREETER, M. D.,
Associate, Wistar Institute of Anatomy.

From the Anatomical Laboratory of Johns Hopkins University.

With 2 Plates and 8 Text Figures.

In the following paper some observations are reported concerning the embryonic morphology of the acoustic nerve and the development of the ganglion mass incorporated in its trunk. The differentiation of this latter mass, the ganglion acusticum, and its subdivision into the ganglion vestibulare and the ganglion spirale present several features of interest; and deserving of especial attention is the additional light which the study of this process throws upon the question of nerve supply of the saccule, and the ampulla of the posterior semi-circular canal. It is found, namely, that these two portions of the membranous labyrinth are not supplied by the cochlear nerve, as described in English and German text books, but are supplied by the vestibular nerve, as has been maintained by some of the French writers. This brings all of the ampullæ together with the utricle and saccule under control of the same nerve, and leaves the cochlear nerve as a specialized and distinct nerve for itself, supplying only the cochlear duct. This arrangement is one which should be gratifying to the physiologist, for it draws a definite line between that portion of the nerve complex which controls the analysis of sound and that which controls equilibrium.

[1] Preliminary reports concerning this investigation were read, and the models demonstrated, at the International Congress of Anatomists at Geneva, August, 1905, and at the meeting of the American Association of Anatomists at Ann Arbor, December, 1905.

This investigation was originally concerned only with the acoustic complex, later it was extended to the ear vesicle, and it was found possible to add several new features concerning the development of this structure and the formation of the membranous labyrinth to that which was already known from the work of His, Jr., 89, who, as far as could be learned, is the only investigator that has made a direct attack on this region in the human embryo since the introduction of wax plate reconstruction methods. It is, of course, to be remembered that in his work attention was mainly directed toward the nerve and ganglion masses, while the finer structure of the ear vesicle was not considered in detail.

The contributions here reported include both additional early stages in the development of the ear vesicle and further details in the formation of the individual parts of the labyrinth. Also some apparently fundamental errors in the work of the above investigator have been here corrected. One of these regards the saccule, which as represented by His, Jr., develops as a compartment pocketing out from the upper end of the cochlea, but which in our specimens develops as a compartment or subdivision of the utricle. Instead of the saccule developing from the cochlea, the cochlea develops from the saccule, though this occurs at a considerable time before the separation between utricle and saccule is complete.

The facial nerve, and especially its sensory division or pars intermedius, bears such a close relation to the auditory apparatus that it was found convenient to include it in some of the reconstructions. It was possible to identify conditions in the embryo confirmatory of what is now the generally accepted opinion as regards the adult, *i. e.,* that the nervus intermedius is the dorsal and sensory root of the seventh, its fibers arising in the geniculate ganglion and continued peripherally in the chorda tympani and great superficial petrosal.

Material and Methods.

This work was made possible through the kindness of Professor Mall, who gave the writer, for the purpose of this investigation, free access to his large collection of human embryos. In the following list are tabulated the embryos which were selected for reconstruction:

List of Embryos Reconstructed.

Number of embryo.	Length in mm.		Probable age in days.	Section.	
	N. B.	V. B.		Thickness.	Direction.
148	4.3	3	20	10 μ	Coronal.
B 17	6.5	6.6	26	15	Sagittal.
2	7	6	26	15	Coron-trans.
163	9	9	30	20	Transverse.
109	10.5	11	33	20	Transverse.
175	13	13	36	20	Transverse.
144	12	14	37	40	Sagittal.
22	18	20	44	50	Transverse.
229	—	21	44	50	Sagittal.
86	20	30	54	50	Coronal.

One or more wax plate reconstructions were made of each embryo after the method of Born. In most cases the models included the membranous labyrinth with the acoustic and facial nerves, and a portion of the central nervous system. Of these models seven were selected for illustration and are shown in Plates I and II. The form of the models has been controlled in all cases by dissections of pig embryos of corresponding stages of development, prepared in the manner described in a previous paper (Streeter, 04, p. 87). Such comparison was of particular assistance in the study of the nerves and ganglion masses. The value of these dissections was greatly increased by previously staining the embryos, in toto, with alum cochineal (powdered cochineal 6 gm., ammonia alum 6 gm., and distilled water 200 cc.), which produces a brilliant differentiation of the tissues. In the same way that a microscopical section is improved by staining so is a stained microscopical dissection that much better than an unstained one. In studying these a strong, direct illumination of the specimen is necessary.

Whenever the size of an embryo is expressed by a single dimension it refers to its greatest length, and the age is that as determined by Mall's rule, *i. e.*, age in days equals the square root of the greatest length times one hundred.

The drawings for Plates I and II were prepared under the guidance and assistance of Mr. Max Brödel, for which the author derives pleasure in taking advantage of this opportunity to acknowledge his appreciation.

Membranous Labyrinth.

The auditory organ is generally described as developing phylogenetically from the lateral line organs of the marine vertebrate, which sink beneath the surface of the body and develop a cartilagenous or bony

capsule, and become incorporated in the underlying head skeleton, the communication with the surface being maintained by a specially devised accessory apparatus.

In the embryo the first sign of the auditory organ, according to Krause, 03, and Poli, 97, consists of a thickening of the ectoderm, the auditory plate, which is seen lateral to the still open medullary groove in the region of the future third brain vesicle. In vertebrates having two layers of ectoderm the thickening involves the inner layer, the outer not being affected. Owing to the fact that the growth of cells shows greater activity in the deeper strata of the auditory plate it soon becomes converted into a cup shape depression and is then called the auditory fossa or auditory cup. By the folding in and closure of its edges the auditory cup is in turn converted into the auditory vesicle, which, however, remains attached to the surface for a longer or shorter period by means of an epithelial stalk or canal being finally separated from the surface, in mammals much earlier than in lower vertebrates.

It is at this point, just after the ear vesicle has been pinched off from the ectoderm, that my own observations begin. This stage corresponds to the "primitive ear vesicle" of Krause, 03, and will be described under that heading here.

The primitive ear vesicle.—The reconstruction of the ear vesicle of an embryo 4.3 mm. long, No. 148, shown in Fig. *a*, Plate I, represents our youngest stage. This is considerably younger than the youngest human embryo described by His, Jr., 89. It is about the same age as shown in Krause's, 03, Fig. 82, a model from a rabbit embryo, and is younger than the first stage of the series of models of the ear vesicle of the bat recently published by Denis, 02.

The ear vesicle consists at this time of a slightly elongated, oval sac, having the following diameters: dorso-ventral, .39 mm.; caudo-cephalic, .26 mm., and transverse, .28 mm. It lies closely against the neural tube, and is connected with it by the acoustic ganglion, similarly as is shown by Mall, 88, in the dog, figured in his Fig. 4, Plate XX, and is surrounded on all sides by a thin layer of mesodermal tissue.

On the dorso-lateral surface, above that portion which is to become vestibular pouch and near where the endolymphatic appendage is to be separated off from the rest of the vesicle, there is a shallow groove. This groove, as seen in the sections, is cut transversely and consists of a seam, or the meeting point of the former edges of the auditory cup whose approximation completes the closure of the vesicle. This closure seam shows various degrees as regards the completeness of fusion, manifested

by a difference in the thickness of the opposite edges, and the degree of obliteration of the line of juncture. The remainder of the vesicle wall is everywhere quite uniform in appearance, consisting of 2-3 layers of slightly elongated epithelial cells, without any apparent differentiation to indicate points of future nerve endings.

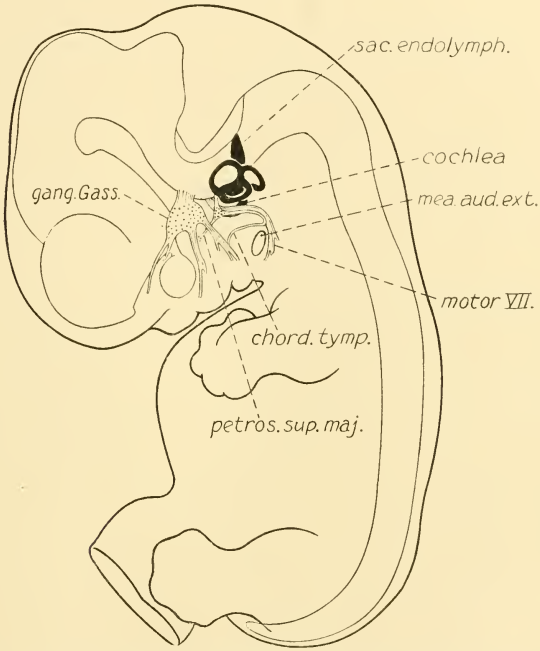


Fig. 1. Profile reconstruction showing the membranous labyrinth and its relative size and relations to the brain and the fifth and seventh cranial nerves. Human embryo 14 mm. long, Mall Collection No. 144, magnified about 8 diams.

No epidermal stalk could be detected connecting the vesicle with the surface, or persisting beneath the surface epithelium, as observed in the rabbit by Krause, 03, p. 88. Evidently in the human embryo such a stalk must be either very temporary or else never present, as here we have to do with a vesicle whose closure and detachment from the surface must be regarded as only just completed.

The development of the endolymphatic appendage and its relation to the epithelial stalk formed during the detachment of the ear vesicle from the epidermis has excited a considerable controversy out of which certain facts have become definitely established. In the first place it is evident (Keibel, 99; Alexander, 01; Krause, 01, and 03) that in the chick the appendage is formed out of the original union region between epidermis and labyrinth anlage, and corresponds to the closing place of the ear vesicle, and is its last point of attachment to the surface. On the other hand it is also established (Corning, 99; Peter, 00, and Krause, 01) that in reptiles and amphibia the tip of the appendage does not coincide with the point of detachment of the ear vesicle, but is situated somewhat more dorsal and proceeds in a course of independent development before the detachment of the vesicle is complete.

In the human embryo the endolymphatic appendage approaches in its development more nearly the type seen in amphibia than that in the chick. It is not developed until the epidermal stalk, if there ever is any such in man, has disappeared. Its anlage is formed by that portion of the vesicle wall just dorsal to the seam of closure, forming a rounded point on the dorsal edge of the vesicle, thus its tip cannot coincide with the point of detachment. Its situation is indicated by the external form before there is any apparent differentiation of the wall and can be seen in Fig. *a,* Plate I. By comparison of Figs. *a-f,* Plate I, it will be noticed how, by a process of extension, this diverticulum becomes converted into the endolymphatic appendage. In the second stage, Figs. *b* and *c,* the external form of the appendage is more distinctly outlined, as a short diverticulum opening widely into the rest of the vesicle. In the next older embryo, Figs. *d, e,* and *f,* by extension of the tip and constriction of its base the appendage begins to assume a typical form. The last step in its differentiation consists in the widening of the distal end into a flattened pouch or sac, in contrast to the remainder, which persists as a narrow duct connecting it with the vestibule, indicated in Figs. *l, m, n,* Plate I, and well marked in *Figs. a, b, c,* Plate II. These are the two divisions of the appendage that are distinguished by the names endolymphatic sac, and endolymphatic duct.

During this process of expansion the wall of the appendage which originally, like the rest of the primitive vesicle, consists of an epithelium of 2-3 layers, is thinned out to a single layer. The thinning out commences in embryos of about 6 mm. It is at first limited to the lateral surface and the extreme tip of the appendage, while the median wall continues to be 2-3 cells thick. It is not until the embryo is about 18

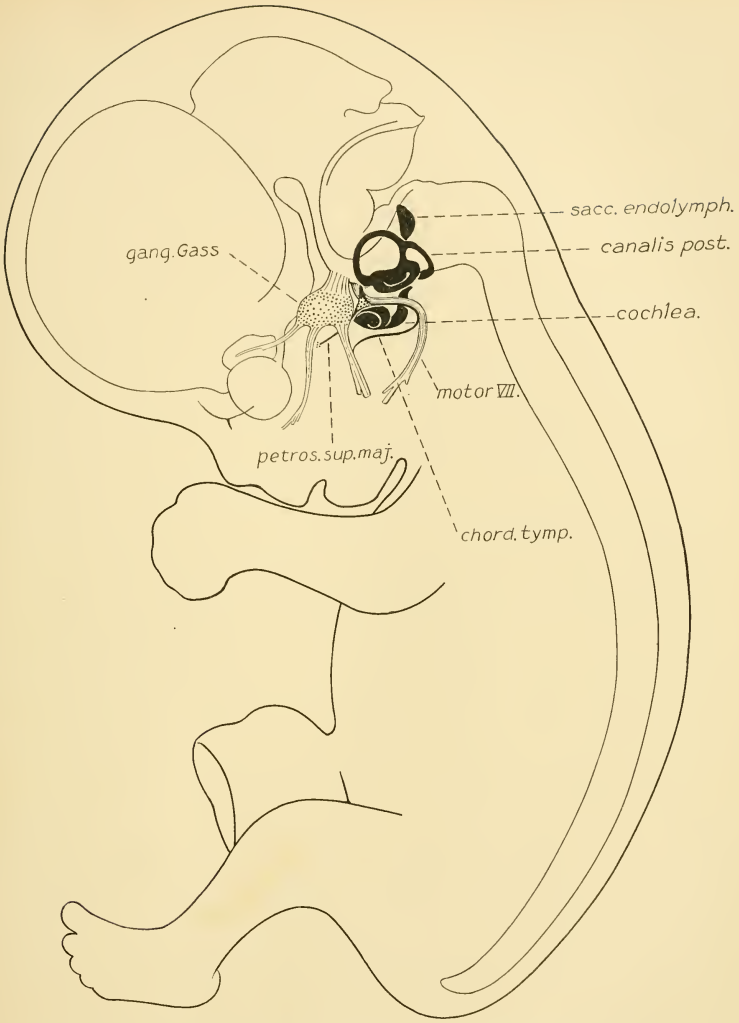


Fig. 2. Profile reconstruction showing the membranous labyrinth and the fifth and seventh cranial nerves. The sensory part of the seventh is indicated by solid black. The great superficial petrosal nerve extends from the geniculate to the spheno-palatine ganglion. Human embryo 30 mm. long, Mall Collection No. 86, magnified about 7 diams.

mm. long that the whole appendage wall is thinned out to a single layer. It seems probable that the thick median wall in embryos of 6-18 mm. constitutes a germinating bed which furnishes the cells needed for the rapidly expanding appendage. It is only these cells that continue to multiply, and they can be imagined as moving around toward the lateral surface in a single layer in the order in which they are derived from their focus of growth.

The diverticulum stage.—Between the primitive vesicle just described and the labyrinth possessing cochlea, semi-circular canals, and accessory recesses, there is a stage through which the ear vesicle passes which can be characterized as the diverticulum or pouch stage. It is represented by the embryos 6.6 mm. and 9 mm. long, shown in Figs. *b-f,* Plate I. In these two embryos the vesicle may be said to consist of two pouches, a large, bulging triangular one above, with the endolymphatic appendage, the vestibular pouch, and opening into it from below the more slender and flattened cochlear pouch. Where these two pouches meet, there is a portion of the vesicle which is destined to form the utricle and saccule. It can be distinctly seen in Fig. *f,* Plate I. This was observed in the bat by Denis, 02, who called that part of it which projects toward the median surface the *diverticule utriculo-sacculaire.* The space concerned, however, involves also a part of the anterior and lateral walls of the vesicle and perhaps it would be advantageous to include this whole region under the concise and descriptive name *atrium.* This atrium is properly a subdivision of the vestibular pouch. It is in fact all that part of it which is left after the separation off of the semicircular canals and their ampullæ. It is not to be confused with the cochlear pouch, which is phylogenetically a secondary diverticulum, which buds out from the atrial portion of the vestibular pouch. The embryonic relation is indicated in the following table:

primary vesicle,	endolymphatic appendage,		endolymphatic duct. endolymphatic sac.
	vestibular pouch,	canal pockets,	semicircular canals. ampullæ.
		atrium,	utricle. saccule.
	cochlear pouch,		cochlea.

If the words pars superior and pars inferior were substituted for the two pouches this conception would then be at variance with Krause, 03, only as regards the saccule which he describes as belonging to the pars inferior. This will be again referred to later.

The surface markings of the vesicle during this stage assume a significant character. In the first place the vestibular pouch at once takes on a triangular shape with the apex toward the appendage. The three borders of this triangle form the anlages of the semicircular canals (see Fig. *d*, Plate I), which bear the same inter-relation as the canals in later stages. A second feature which is apparently constant and important is the sharp, vertical groove, which cuts in between the anlage of the posterior canal and the posterior end of the lateral canal. This we may call the *lateral groove*. It was not represented by His, Jr., 89, but can be seen in the model from the 8 mm. rabbit of Krause, 90, p. 296, and still better in models 3, 4, and 5 of Denis, 02, which were taken from the bat. The latter author mentions it in his text.

Ventral to the anlage of the lateral canal, on the lateral surface of the vesicle there is a rather large depression or fossa, which becomes more marked in proportion to the increasing projection of the lateral canal, which overhangs it like a shelf. This fossa forms the lateral wall of the atrium from which the utricle and saccule are to develop. The cochlear portion of the vesicle is limited to its ventral tip and extends up along the rounded posterior border nearly to the prominent anlage of the posterior canal. There intervenes between them that portion of the wall that is to become the posterior ampulla. The tip of the cochlea begins to bend forward practically as soon as the cochlear pouch can be distinguished as such.

The changes in the structure of the wall of the ear vesicle which accompany the pouch formation are limited to the thinning out of certain areas on the dorso-lateral surface of the vestibular pouch, and the lateral surface of the appendage as has already been referred to. The remainder of the vesicle wall is of the primitive type; there were no areas that could be recognized as nerve endings. In embryo No. 163, 9 mm. long, however, protoplasmic nerve processes extend from the ganglion and lose themselves in the vesicle epithelium. The branch destined to become the posterior ampulla nerve could be seen with great distinctness; but where it ended there was no reaction to be seen on the part of the epithelium.

The period of *semicircular canal formation* is shown in Figs. *g-k*, Plate I. The process consists in the expansion of the edges of the vestibular pouch, *i. e.*, the canal anlages, and the coincident absorption of the intermediate vestibular walls, as was essentially described by Böttcher in his monumental work of 1869, and to some extent by other observers even previous to that. Since then further details have been worked out

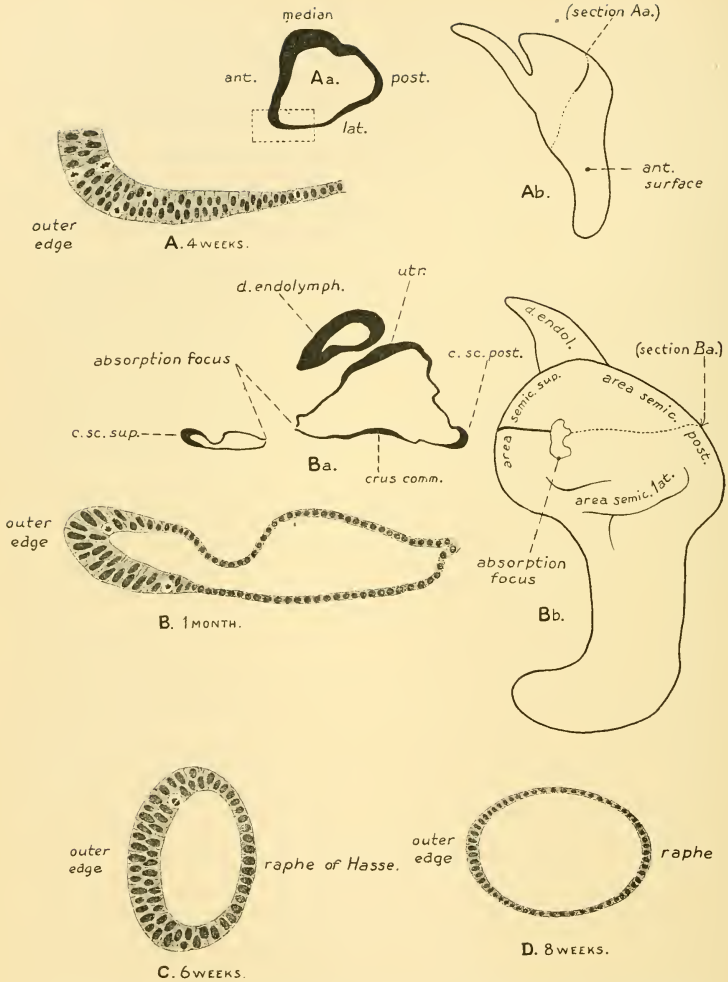


FIG. 3. Development of a semicircular canal. *A*, *B*, *C*, and *D* represent transverse sections of the superior canal (x 240), taken at corresponding points from embryos No. 163, 9 mm.; No. 109, 11 mm.; No. 229, 21 mm., and No. 86, 30 mm. *Aa*, *Ab*, *Ba*, and *Bb* are explanatory drawings of lower magnification to show the ear vesicle and the situation and shape of sections from which *A* and *B* are taken.

by various investigators, notably by Krause, 90, and 03, who approached the problem along the whole line of vertebrates. He demonstrated that the canals are formed one after the other in definite sequence, the superior first then the posterior and lastly the lateral. Our information concerning the human semicircular canals is based principally on the work of His, Jr., 89.

An interesting interval, which was left open by His, Jr., between his stages shown in his Figs. 6 and 7, Plate I, is filled in by my models, made from 11 and 13 mm. embryos (Figs. *g-k*, Plate I). What is to be particularly noted is the change occurring in the structure of the vestibular wall which can be seen from a surface examination of the model. Those areas which are to persist stand out prominently and present a fairly definite outline of the future labyrinth, while the intermediate areas, which are destined to be absorbed, collapse before the advancing mesoderm; this is well shown in Figs. *j* and *k*. It might be thought that the absorption of epithelium in Fig. *j* had been completed as far as the superior canal is concerned, and that the remaining epithelium would go to make the canal wall, necessarily stretching out to obtain the diameter represented by the same canal in Fig. *m*. This, however, is not the case; it is only the thickened edge of the pockets of the vestibular pouch that becomes canal wall. In Fig. *j* there still remains a large area of epithelium that is to be absorbed before the inner rim of the superior canal is reached.

The histogenesis of the semicircular canal is shown in the accompanying Text Fig. 3, in which *A, B, C,* and *D* represent transverse sections of the superior canal in four stages of differentiation, taken at corresponding points and magnified the same number of diameters. The striking feature of the process is the persistence in the canal anlage of the primitive epithelium of 2-3 layers until after the canal is closed off, evidently being a factor in its rapid growth. Section *A* is taken from the ear vesicle of a 9 mm. embryo, the same as shown in Figs. *d, e, f,* Plate I. *Aa* shows the entire section of which *A* is a portion, and *Ab* indicates the direction of the section as regards the ear vesicle. Section *B* is from a 11 mm. embryo. The entire section is represented by *Ba,* whose position as regards the ear vesicle is shown on *Bb,* which is from the same model shown in Figs. *h, i, j,* Plate I. Sections through the vestibular region at this stage are very interesting, as they show by the thickness of the wall which are the persistent areas; section *Ba* is made in such a way as to include the anlages of two canals, the lateral wall of the crus commune and a part of the utricle and the ductus

endolymphaticus, all of which stand out prominently. The intervening vestibular epithelium, which is doomed to absorption, consists of a single layer of cuboidal cells, as shown in *B*, in contrast to the thick outer edge which is to become canal.

This process of absorption may be described histologically as a conversion of the definite epithelial membrane into a line of cells which seem to fuse with and cannot easily be distinguished from the adjacent mesodermal cells, the line finally becoming broken and irregular. The transition from one step in this procedure to the next is quite abrupt; thus in *B* the thin membrane is sharply cut off from the absorption focus. Several specimens were examined of about this age, and in one case, embryo No. 175, 13 mm. long, it was found that absorption of the epithelium was going on before the lateral and median walls of the vesicle had actually come together. So it is possible that during this process the vesicle cavity is in some cases left temporarily in open communication with the spaces of the adjacent mesoderm. The final curling in of the edges and closure of the canal tube repeats in a way the procedure which we have already seen in case of the auditory cup during its conversion into the auditory vesicle. It is probably likewise mechanically brought about by the arrangement of the epithelial cells. Section *C* shows the canal after the formation of the closure seam, the so-called raphe of Hasse. The thickness of the epithelium of the outer edge and presence of division figures indicate that the activity of growth still continues. Section *D* shows a canal in an embryo 30 mm. long, the same stage as that shown in Figs. *a, b, c,* Plate II. Here the epithelium is reduced to a single layer and division figures have disappeared. It can be seen, however, that traces still exist of the thickened outer edge and the raphe of Hasse. This stage differs from the adult canal practically only in its diameter, which there is 3-4 times greater. Doubtless this growth is in large part accomplished simply by the flattening out and expansion of the individual cells.

The formation of the ampullæ can be seen by comparing the figures on Plates I and II. It will be noticed that their development proceeds simultaneously with that of the canals. In their histogenesis they resemble the canals, in having a thin single layer of epithelium on the inner rim and the thick 2-3 layered epithelium on the outer surface. It is out of the latter primitive epithelium that the maculæ are developed, and they make their appearance before ampullæ and canals are completely separated from the remainder of the vestibular sac; they can be seen in the 11 mm. stage, but a high degree of differentiation is not

found until we come to embryos 20 mm. long. It will be remembered that His, Jr., 89, represents ampullæ as forming on both ends of the superior and posterior canals. This was not confirmed in our models; the ends of these two canals where they unite to form the crus commune show no such enlargement. Each canal possesses but one ampulla.

The *development of the utricle and saccule* is dependent on the subdivision of the atrium into an upper and lower compartment. The atrium, as has already been described is that ventral part of the vestibular pouch into which the endolymphatic appendage opens, and into which the cochlear pouch opens from below; in Fig. *f*, Plate I, it is marked utric-sacc., and in Fig. *j* the lateral surface of it is marked sacc., and in Fig. *k* a partial median view of it is marked utric. In Figs. *j* and *k*, though the canals and ampullæ are already completing their separation from the vestibular pouch, the atrial region has not yet begun its subdivision. It, however, suggests by its outer form the future saccule and utricle. The actual subdivision begins in embryos between 18 and 20 mm. The initial ingrowth of the membranous partition can be seen in Figs. *l* and *m*, where it can be distinguished as a horizontal cleft which forms in front between the utricular and saccular parts of the atrium. Strictly speaking we cannot speak of a saccule and utricle until the intervening partition is complete. It is practically complete in Figs. *a*, *b*, *c*, Plate II; here it reaches back to the entrance of the ductus endolymphaticus. It later divides the orifice of that structure, thus affording it separate openings into the utricle and saccule, the two openings constituting the so-called ductus utriculo-sacculares.

In the meantime the utricle itself has developed a definite shape. As can be seen in the Figs. *a*, *b*, and *c*, a transverse constriction divides it into an anterior or cephalic part and a posterior or caudal part. The anterior part constitutes the general utricular cavity, in the floor of which the nerve ends. In front, just ventro-median to the ampulla of the superior canal, a distinct diverticulum extends forward from it which is called the recessus utricularis. The posterior part consists of a central sinus utriculi communis, into which opens from above the crus commune, laterally the sinus utriculi lateralis of the lateral canal, from below the sinus utriculi inferioris of the posterior canal, and on the median side the ductus endolymphaticus.

If one compares Figs. *a*, *b*, *c*, Plate II, with pictures of adult preparations such as found in the beautiful atlas of Schönemann, 04, it is apparent that the labyrinth of the 30 mm. embryo has practically completed its gross development. In its further expansion all parts of it

become relatively more slender and the saccule draws away from the utricle and becomes flattened as well as biconcaved or saucer-shaped.

The *cochlea* as compared with the derivatives of the vestibular part of the ear vesicle is less complicated in its development, presenting only the peculiarity of spiral growth. The cochlea has already been referred to as the pouch which forms the ventral tip and part of the posterior border of the vesicle, as seen in Figs. *b-f*. In Figs. *g, k,* it is partly demarcated from the saccular region by a broad fossa. At 20 mm., Fig. *l,* a sharp constriction separates it from the saccule, and this becomes in the 30 mm. embryo the ductus reuniens, and in the meantime the cochlea has become a spiral of two turns.

As regards the relation of cochlea to saccule we differ from the description given by His, Jr., 89, who represents the saccule as budding off from the upper end of the cochlea, which is just the reverse of our own interpretation and what might be expected on the ground of the comparative anatomy of these structures. We know that in certain fishes the ear vesicle consists of a simple utricle into which the semicircular canals empty. In certain other fishes pockets bud out from the utricle analogous to the saccule. When we come to animals that leave the water, the amphibians, there develops from the saccule a secondary pocket, which in birds and reptiles takes on the characteristics which identify it with the mammalian cochlea. That is to say, first utricle, then utricle and saccule, and finally utricle, saccule, and cochlea. The phylogenetic development presents here, in discrete steps, the process which we find in the human embryo, but in the latter case it is a matter of simultaneous growth of all three structures.

A *resumé* of the development of the labyrinth is presented in the form of a diagram in the adjacent Fig. 4, which illustrates the successive steps by which the simple ear vesicle enlarges and becomes differentiated into the group of connected individual compartments which characterize the adult ear.

The ear vesicle very early (6-7 mm. long, 3½ weeks) assumes the form of two communicating pouches, the vestibular pouch, with its endolymphatic appendage, and the cochlear pouch. The first gives origin to the semicircular canals, ampullæ, utricle, and saccule. The semicircular canals, in consequence of the approximation and absorption of the intervening wall of the vesicle, make their appearance between the fourth and fifth weeks, 9-14 mm. (only one is shown in the diagrams). That portion of the vestibular pouch that is not involved in the formation of the canals and their ampullæ may be called atrium, to indicate that

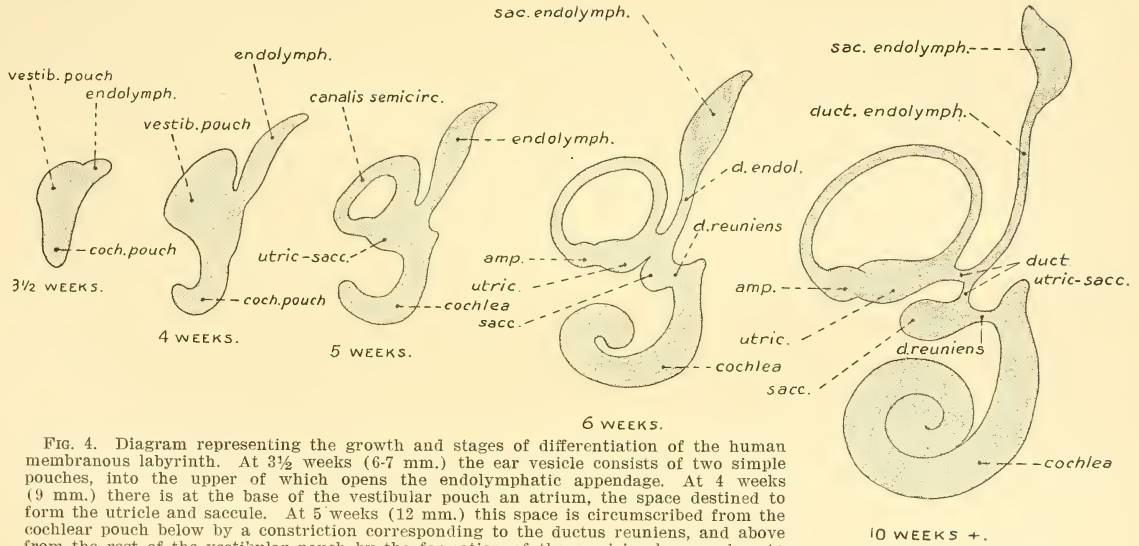


FIG. 4. Diagram representing the growth and stages of differentiation of the human membranous labyrinth. At 3½ weeks (6-7 mm.) the ear vesicle consists of two simple pouches, into the upper of which opens the endolymphatic appendage. At 4 weeks (9 mm.) there is at the base of the vestibular pouch an atrium, the space destined to form the utricle and saccule. At 5 weeks (12 mm.) this space is circumscribed from the cochlear pouch below by a constriction corresponding to the ductus reuniens, and above from the rest of the vestibular pouch by the formation of the semicircular canals. At 6 weeks (20 mm.) an ingrowth of the wall of the atrium divides it into an upper part (utricle) and lower part (saccule). At 10 weeks (30 mm.) this partition between the utricle and saccule is complete and extends inward in such a way as to split the orifice of the endolymphatic duct.

it forms at this time a common meeting place into which open the different compartments, including the endolymphatic appendage. At six weeks, 20 mm., the atrium becomes separated into an upper and lower division by an ingrowth of its wall, thus forming the utricle and saccule. This partition continues inward in such a way as to split the orifice of the ductus endolymphaticus, the divided ends of which form the ductus utriculo-sacculus. The cochlear pouch opens directly into the atrium, and as the development proceeds it can be seen that it is into that part of the atrium which is destined to form the saccule. At the fifth week, 14 mm., a beginning constriction appears between the cochlea and the saccular region. This constriction corresponds to the ductus reuniens and gradually narrows down until in the adult in many cases the communication between cochlea and saccule is obliterated. It is very apparent that the saccule is not developed from the cochlea, but the cochlea may be said in a certain sense to develop from the saccule.

N. Vestibularis and N. Cochlearis.

The earlier anatomists described the auditory nerve as being made up of two main divisions. One of these, according to their plan, supplied the utricle, saccule, and the ampullæ of the three semicircular canals, while the other division they considered to belong exclusively to the cochlea. This description prevailed up to the time the exhaustive monograph was published by Retzius, 84, upon the comparative anatomy of the membranous labyrinth and its nerves. This investigator, by means of careful dissection of a great variety of vertebrate material, was able to present a much more minute description of the n. acusticus than had previously existed. In mammals, according to his view, the anterior division or ramus vestibularis supplied the utricle, and the superior and lateral ampullæ, while the posterior division or ramus cochlearis supplied the saccule, the posterior ampulla and the cochlea. This classification was substantiated not long after by His, Jr., 89, in his paper on the development of the human acoustic complex, in which he also represented the cochlear division as supplying not alone the ductus cochlearis but also the saccule and ampulla of the posterior canal. From that time until now the classification made by Retzius has been the one generally adopted by both English and German text books. Certain French writers (Cannieu, 94, 04, and Cunco, 99), however, have come back to the original conception of the cochlear nerve and its individuality. They point out that Retzius fuses in his ramus cochlearis the inferior branch of the ramus vestibularis and the cochlear nerve proper. They admit

that these two lie side by side and are closely united, but further than that deny any anatomical or physiological relation. A similar conclusion has also been reached by Alexander, 99, who studied serial sections of the acoustic ganglion mass taken from various adult mammals.

My own observations concerning the development of these structures in the human embryo are quite contrary to those of His, Jr., and as will be immediately seen, they seem to indicate that the cochlear division of this complex has nothing to do with the nerves to the saccule and posterior ampulla, but possesses its own specialized characteristics which distinguish it from all the rest of the acoustic mass. Embryologically, therefore, it seems well to follow Cannieu's, 94, lead and adopt the following classification:

N. Octavus (N. Acusticus).

- n. vestibularis,
  - pars superior,
    - r. ampul. sup.
    - r. ampul. ext.
    - r. recess. utric.
  - pars inferior,
    - r. sacc.
    - r. amp. post.
- n. cochlearis,
  - ramuli spirali.

The form and branches of the acoustic mass in its different stages and its relation to the labyrinth is shown in the figures on Plate I and II. Two colors are added so that the cochlear division can be distinguished from the vestibular; the former is colored yellow and the latter light red. The same ganglion mass is shown more diagrammatically in the accompanying Fig. 5, showing its appearance in embryos 4, 7, 9, 20, and 30 mm. long. The vestibular part is indicated by fine dots and the cochlear by coarse dots. The drawings on the left present a median view and those on the right a lateral view.

In the youngest stage, embryos of about 4 mm., Mall collection, No. 148, the outlines of the ganglion mass are indefinite, particularly the peripheral border. The central end is more distinct and the protoplasmic cell processes can be seen leading to the wall of the neural tube. This is somewhat younger than the earliest stage of His, Jr., 89. In the next stage, embryos of about 7 mm., the outlines of the ganglion can be clearly made out. A section through such a ganglion is shown in Fig. 6. It lies closely against the front edge of the vesicle, its lower end migrating around on the median side. In its outer form it consists of an upper and lower part, pars superior and pars inferior, each of which develops its own separate group of peripheral nerve branches; the central root

of the ganglion connecting it with the brain consists of a single stem. Owing to the proximity of the ganglion mass to the ear vesicle the nerves uniting them are at this time very short. His, Jr., 89, p. 6, regards that portion of the ganglion which we have called the pars inferior as the ganglion cochleare. What I regard as the ganglion cochleare or ganglion spirale does not make its appearance until a trifle later, in embryos of about 9 mm. There can be seen then a group of ganglion cells massing themselves on the ventral border of the pars inferior, which corresponds completely to the future spiral ganglion and may be considered as its anlage. This anlage develops into a derivative which buds off from the pars inferior and then follows an individual course of growth independent of the latter, and this is analogous to the way in which we have already seen the membranous cochlea bud off from the saccule and develop independently.

That part of the pars inferior which does not participate in the formation of the spiral ganglion remains closely related to the pars superior, and supplies the saccule and posterior ampulla. It is this that His, Jr., describes in a later stage as the *Zwischenganglion,* and whose centripetal fibers he joins to those of the main cochlear trunk.

In embryos of 20 mm. (compare Figs. *l, m, n,* Plate I) the pars superior has increased greatly in size, and its peripheral nerves, which before were massed together, have become separate and distinct branches. The pars inferior, from which the spiral ganglion is rapidly separating, consists of a connecting strand of ganglion cells giving off separated branches to the saccule and posterior ampulla. The fibers extending to the posterior ampulla are at first (embryos of 11 mm.) loosely spread out and give the appearance of more than one nerve, but later, either by atrophy of some of them or by becoming bundled together more closely, they constitute a single compact nerve. It is possible that here we have to do with temporary fibers representing branches to the additional nerve endings which are found in this region in lower forms.

The cochlear nerve can be distinctly seen collecting its fibers from the spiral ganglion and extending up toward the brain. The exact manner in which this nerve reaches the neural tube proved difficult to determine. It apparently sprouts out from the spiral ganglion and travels up on the median surface of the vestibular ganglion until it reaches the brain. To be certain of this would require a greater number of stages between 8 and 10 mm. than were available. In the embryos studied the proximal end of the nerve could be made out almost as soon as the distal. So it is possible that the cochlear trunk consists originally of a column of

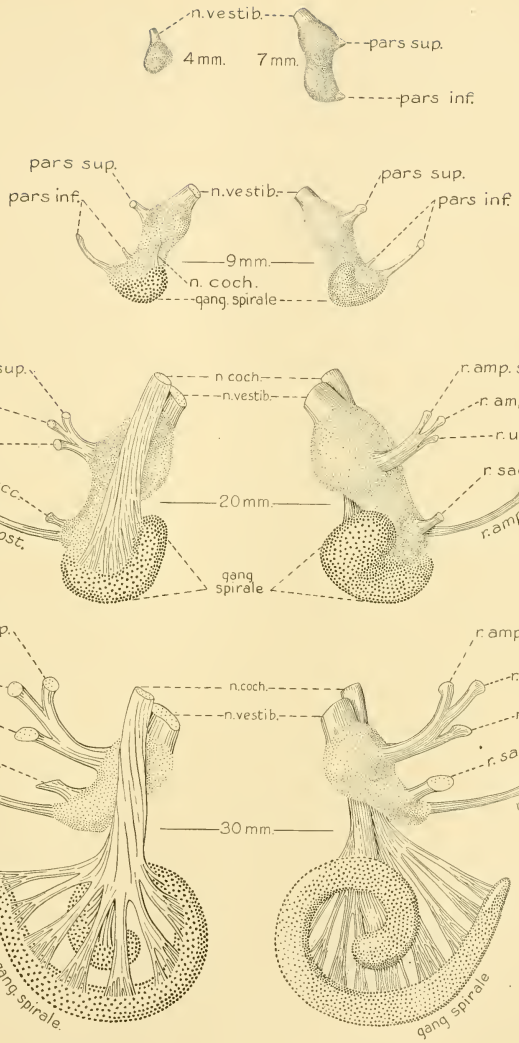


FIG. 5. Stages in the differentiation of the acoustic nerve complex. Vestibular ganglion shown by fine dots, and spiral ganglion by large dots.

ganglion cells connecting the anlage of the spiral ganglion with the brain, and the conversion of this column into fibroblasts produces the early fibers of the trunk; this would explain the abrupt appearance of the nerve trunk in all parts of its course at once.

Proceeding to embryos 30 mm. long, the same as seen in Figs. *a, b, c,* Plate I, we meet with conditions which are practically those found in the adult. There is the vestibular nerve, on whose trunk is situated its ganglion mass, consisting of an upper and lower division. The upper division is connected with the labyrinth by the branches supplying the anterior and lateral ampullæ, and the utricle; the lower division gives off branches to the sacculus and posterior ampulla. In the adult the division between the pars superior and pars inferior is still more pronounced and the separation can be even seen in the trunk of the nerve. The ganglion mass is completely divided except for a bundle of anastomosing fibers, which according to Alexander, 99, may also be accompanied by a chain of ganglion cells—a persistence of the embryonic connection between the pars superior and pars inferior.

The cochlear nerve lies on the median surface of the two divisions of the vestibular ganglion, but is connected with them only by contiguity. This can be demonstrated by dissection methods in pig embryos; the cochlear trunk can be easily lifted off, leaving the vestibular ganglion mass and all of its branches in position, both of the pars inferior and pars superior. At the point where they enter the central nervous system the cochlear and vestibular trunks are in the 30 mm. embryo closely united; they run into each other slightly more than is shown in Fig. 5, and it is not easy to distinguish at what point the one stops and other begins. Their separation is brought about by the increase in size of the restiform body which develops in between them. During this process it could easily happen that some of the cochlear fibers should become grouped in with the vestibular, or that some of the vestibular should become grouped in with the cochlear; in both cases finally reaching their proper destination. If the individual fibers were traced in a large number of cases undoubtedly a considerable variation in this respect would be found. An important stride in this direction was made by Held, 93.

The twisted, rope-like character of the cochlear nerve, as indicated in Fig. 5, is easily identified in the 30 mm. embryo. An effort was made in the pig to determine the number of turns and their relation to the number of turns of the spiral ganglion. There is apparently a certain amount of correspondence, but the fibers were too tightly adherent to admit of a satisfactory unrolling of the nerve.

In the summary of these nerves it should be emphasized that we have distinct points of difference between the vestibular and cochlear divisions of the acoustic complex, both as regards the ganglia and the nerve trunks themselves. The ganglia, belonging to the vestibular division, are spread along the trunk of the nerve; the ganglion of the cochlear division is situated at the extreme distal end of the nerve, and lies directly on the membranous labyrinth, being closely incorporated with it later in the cartilagenous capsule. The vestibular terminal branches develop as discrete and fairly long nerves; the cochlear terminal branches are short and freely anastomose. The main trunk of the cochlear division is characterized by the compactness of its fibers and their spiral arrangement; while in the vestibular division the fibers are less compactly bundled, showing a tendency to subdivision, and do not have the spiral character.

N. Facialis and Pars Intermedius.

The facial nerve is so closely united in position with the acoustic ganglion that it was found advisable to include it in the reconstructions. For reason of simplicity it is not shown in Plates I and II, but its form and general position at two different stages is shown in the Text Figs. 1 and 2. It can be seen how it is divided into ventral and dorsal (motor and sensory) roots, and the situation of the geniculate ganglion on the latter.

Particular attention was given to the ganglion geniculatum and pars intermedius in order to bring their early morphology into accord with the conditions found in the adult. Though the "portio media inter communicantem faciei et nervum auditorium" was described by several writers over a century ago, and in the intervening time frequent reference and much speculation has been made concerning it; yet it has turned out that none of this was actually in advance of the original description until there appeared the paper of Sapolini, **83**, who was the first to give a detailed report of its deep origin and terminal distribution. This investigator succeeded in dissecting out the pars intermedius in adult human material throughout its whole course. He describes it as arising in the floor of the fourth ventricle, from where it ascends as a nerve band which runs along the median edge of the acoustic area. At the level of the inferior cerebellar peduncles it makes its exit through the side of the pons, between the seventh and eighth cranial nerves, and then passes through the geniculate ganglion, and is continued into the chorda tympani, which in turn joins the lingual branch of the fifth nerve and forms

a terminal plexus in the substance of the tongue. We are thus indebted to this writer for establishing the fact that the nerve of Wrisberg, or pars intermedius, and the chorda tympani, are two continuous parts of the same nerve, connecting the anterior part of the tongue with the floor of the fourth ventricle, an essential fact which has been very slow in getting into our text books. Further details regarding the central path of this nerve is given in a supplementary note in a paper of His, 90, in description of a 17 mm. human embryo. He reports this nerve as extending from the geniculate ganglion as an independent bundle into the brain, where it can be seen running along the median edge of the acoustic area spinalwards until it reaches the entering fibers of the

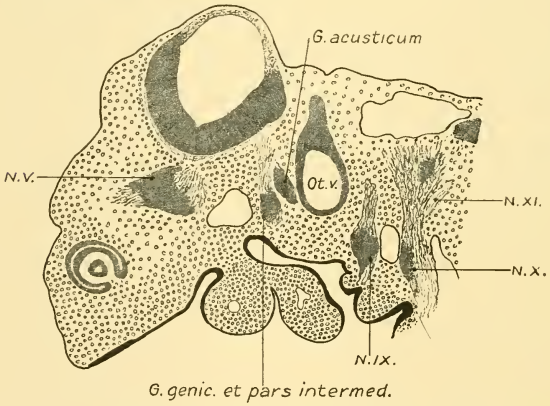


FIG. 6. Sagittal section of a 7 mm. human embryo (B. 17), showing the relations of the facial-acoustic complex.

glossopharyngeus. It joins with these fibers and together with them takes part in the formation of the tractus solitarius. His felt justified in assuming that we have here to deal with a union within the neural tube of the taste fibers from the anterior and posterior portions of the tongue, one group coming through the chorda tympani and nerve of Wrisberg, and the other through the glossopharyngeus. This view has since then been vigorously supported by Dixon, 99, who pictures the facial nerve as a typical branchial nerve developed in connection with the ear cleft, having a motor part behind the cleft supplying the muscles developed from this arch, and a sensory part made up of the chorda tympani and great superficial petrosal which consist almost entirely of

taste fibers. Reasoning from the development and comparative anatomy of the chorda tympani he points out the improbability of the presence of any taste fibers in the trigeminal nerve as had been maintained by many. That the fifth nerve pair contains few or no taste fibers has been further established by the careful observations of Cushing, 04, who, after the removal of the Gasserian ganglion, fund in all of his cases that the anterior part of the tongue retained in greater or less degree the sense of taste as well as common sensation.

If after the work of Sapolini there remained still doubt regarding the anatomical identity of the pars intermedius, geniculate ganglion, and chorda tympani, it was removed by the convincing dissections of Penso, 93. This worker investigated these structures in man and a considerable variety of other mammals, using principally dissection and teasing methods, though his observations were made also in part from serial

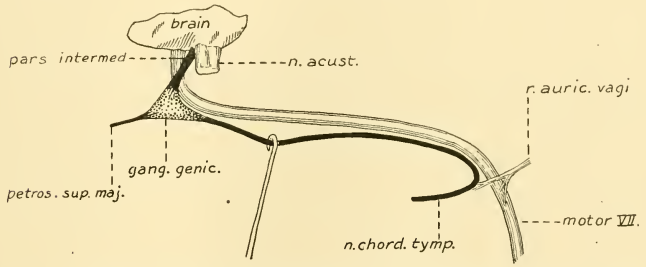


Fig. 7. Drawing made from a dissection of the facial nerve in a 20 cm. pig embryo. At this stage the sensory and motor divisions can be easily separated.

sections. He showed conclusively that the geniculate ganglion is primarily connected with the motor trunk of the facial only by contiguity, its fundamental attachments being a central one, the pars intermedius, and two peripheral ones, the chorda tympani and the great superficial petrosal. He also describes a number of small anastomoses existing between these branches and the surrounding structures, including the motor portion of the facial, the acoustic ganglion, the spheno-palatine ganglion, and the auricular branch of the vagus. He therefore represents the great superficial petrosal and chorda tympani as composite nerves, which are made up, in the first place of fibers from the geniculate ganglion, and in addition to these the fibers from the above-mentioned anastomosing branches. During the past year a paper confirming Penso's work in its

essential points has been published by Weigner, 05, who studied serial sections of rodent and human material, and found it possible to trace to their destination the fibers of the n. intermedius by their histological character; that is to say, from the frequency of the sheath nuclei, the small size of the fibers, and the presence of scattered ganglion cells lying along the course of the fibers.

The common identity of the pars intermedius geniculate ganglion and chorda tympani, as described by Sapolini and Penso, in the adult is less easily seen in the early embryo owing to the incomplete differentiation of these structures. In fact, His, Jr., 89, states that up to the third month no nerve is to be seen arising between facial and acoustic, and he concludes that the intermedius must until that time run in the trunks of these two nerves. In our embryos, however, it was found earlier than that. The Fig. 6 represents the pars intermedius and geniculate ganglion in an embryo of 3½ weeks, and they can also be dis-

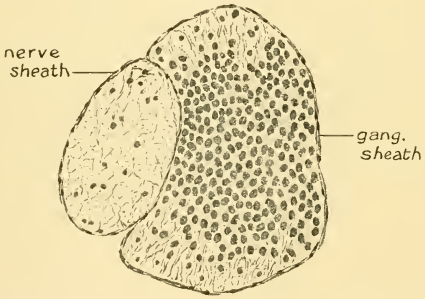


Fig. 8. Sagittal section through the geniculate ganglion and facial nerve of a 30 mm. human embryo, No. 75, Mall Collection, showing how they are separated by a connective tissue partition.

tinguished in embryo No. 148, Mall collection, which is about 20 days old. The ganglion can be made out first, and shortly after that the path of loose fibers connecting it with the neural tube. As is seen in Fig. 6, the ganglion and its proximal root are at this early period distinctly separate from the acoustic mass, and it is only as the acoustic mass increases in size that it could be said to fuse with the geniculate ganglion; the existence even here of a real fusion is doubted, for no appearance was observed in any of our embryos that could not be explained by mere contiguity. The separation existing between these ganglia, in the early stages, merits the attention of those who would

believe that they have a common origin, as was thought by the younger His.

In Fig. 6 the ventral or motor division of the facial nerve can be seen cut obliquely at the ventral edge of the geniculate ganglion. In this embryo the dorsal root or pars intermedius is fully as large as the ventral or motor root; but the proportion is gradually reversed as one looks through older stages, due to the more rapid growth of the ventral root. The pars intermedius is in this sense a more prominent structure in fœtal life than in the adult, indicating that phylogenetically it has played a more important role in lower forms than in man. With the development of the connective tissue the geniculate ganglion becomes inclosed in a sheath, and is walled off from the motor root, against which it continues to lie, as may be seen in Fig. 8. In this figure the ganglion is cut somewhat obliquely, and at the two ends can be seen the central and peripheral fibers of the ganglion. To determine the destination of the peripheral fibers dissections were made of embryonic pigs and it was found easy to demonstrate in 3-20 cm. embryos that the distal fibers leave at one corner of the ganglion by the great superficial petrosal nerve and at the other by a bundle that runs along the motor division until it leaves it as the chorda tympani. Fig. 7 represents such a dissection made in a 20 cm. pig, showing the nature of the anastomosis with the auricular branch of the vagus, and following essentially the arrangement described by Sapolini and Penso.

In these dissections of the seventh nerve of pig embryos a ganglion cell mass, connected with the pons ganglia, was seen extending caudalwards as a surface ridge which could be traced beginning at the fifth nerve and then passing in between the seventh and eighth nerves and finally ending on the dorso-lateral surface of the restiform body. This is apparently the same structure that has been found by Mr. C. R. Essick, of the Johns Hopkins Medical School, in human adult material, and a full description of which he has now ready for publication.

REFERENCES.

Alexander, G., 99.—Zur Anatomie des Ganglion vestibulare der Säugethiere. Sitz. d. Kais. Akad. d. Wiss., math.-naturw. Classe, Bd. 108, Abth. III, 1899.

——— 01.—Zur Entwicklung des Ductus endolymphaticus. Arch. f. Ohrenheilkunde, Bd. 52.

Böttcher, A., 69.—Ueber Entwick. u. Bau des Gehörlabyrinthes. Verhandl. d. Kaiserl. Leop. Carol. deutschen Akad. der Naturforscher., Bd. 35.

Cannieu, A., 94.—Recherches sur le nerf auditif. Thèse doctorat en medicine. Bordeaux. (Quoted from Cannieu, 04.)

——— 04.—Oreille interne. Traité d'anatomie humaine. Poirier et Charpy, Paris, Tome V.

Corning, H. K., 99.—Ueber einige Entwicklungsvorgänge am Kopfe der Anuren. Morph. Jahrb., Bd. 27.

Cunéo, B., 99.—Nerfs craniens. Traité d'anatomie humaine. Poirier et Charpy, Paris.

Cushing, H., 04.—The sensory distribution of the fifth cranial nerve. Johns Hopkins Hospital Bulletin, Vol. XV.

Denis, P., 02.—Recherches sur le developpement de l'oreille interne chez les mammifères. Arch. d. Biol., Vol. XVIII.

Dixon, A. F., 99.—The sensory distribution of the facial nerve in man. Jour. of Anat. and Physiol., Vol. XXXIII.

Held, H., 93.—Die centrale Gehörleitung. Arch. f. Anat. und Physiol., Anat. Abth.

His, W., 90.—Entwickelung des menschlichen Rautenhirns. Abhandl. d. S. Kgl. Sächs. Gesell. d. Wiss., Bd. 17.

His, W., Jun., 89.—Zur Entwickelungsgeschichte des Acustico-Facialgebietes beim Menschen. Arch. f. Anat. u. Physiol. Anat. Abth. Suppl.

Keibel, F., 99.—Ueber die Entwicklung des Labyrinthanhanges. Anat. Anz., Bd. 16.

Krause, R., 90.—Entwicklungsgeschichte der häutigen Bogengänge. Arch. f. mikr. Anat., Bd. 35.

——— 01.—Die Entwickelung des Aquæductus vestibuli s. Ductus endolymphaticus. Anat. Anz., Bd. 19.

——— 03.—Entwickelungsgeschichte des Gehörorgans. Handbuch der Entwickelungslehre, Lief. 4 and 5, O. Hertwig, Jena.

Mall, F. P., 88.—The branchial clefts of the dog. Studies from Biolog. Lab. of Johns Hopkins Univ., Baltimore.

Penso, R., 93.—Ueber das Gang. geniculi u. die mit demselben zusammenhängenden Nerven. Anat. Anz. 8.

Peter, K., 00.—Der Schluss des ohrgrübschens der Eidechse. Arch. f. Ohrenheilk., Bd. 51.

Poli, C., 97.—Zur Entwickelung der Gehörblase bei den Wirbelthieren. Arch. f. mikr. Anat., Bd. 48.

Retzius, G., 84.—Das Gehörorgan der Wirbeltiere. Stockholm.

Sapolini, 83.—Etudes anatomiques sur le nerf de Wrisberg et la corde du tympan on un treizième nerf cranien. Journ. de Med. de Bruxelles. Vol. LXXVII.

Schönemann, A., 04.—Die Topographie des Menschlichen Gehörorganes. Wiesbaden.

Streeter, G. L., 04.—The development of the cranial and spinal nerves in the occipital region of the human embryo. Amer. Journ. of Anat., Vol. IV.

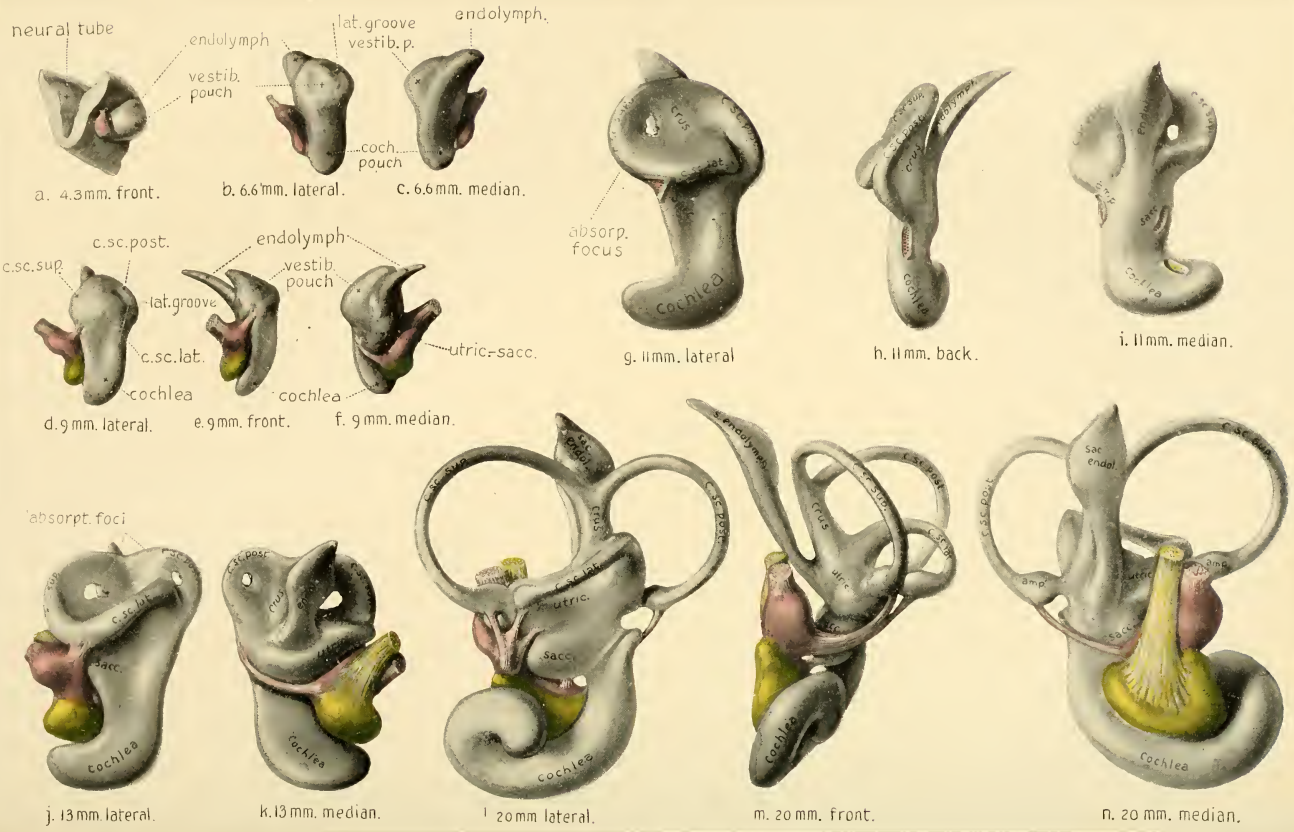
Weigner, K., 05.—Ueber den Verlauf des Nervus intermedius. Anat. Hefte. 87.

DESCRIPTION OF PLATES I AND II.

The reproductions shown on these plates represent different views, in most cases lateral, front, and median, of seven selected models showing the membranous labyrinth and acoustic complex reconstructed from the following human embryos: No. 148, 4.3 mm.; No. B17, 6.6 mm.; No. 163, 9 mm.; No. 109, 11 mm.; No. 175, 13 mm.; No. 22, 20 mm.; No. 86, 30 mm. The colors, yellow and red, are used to indicate respectively the cochlear and vestibular divisions, and in general nerve fibers can be distinguished from ganglion cell masses by their lighter tone. The pictures represent a magnification of 25 diams.

The following abbreviations are used:

absorpt. focus = area of wall where absorption is complete.
amp. = ampulla membranacea.
crus = crus commune.
c. sc. lat. = ductus semicircularis lateralis.
c. sc. post. = ductus semicircularis posterior.
c. sc. sup. = ductus semicircularis superior.
coch. or cochlea = ductus cochlearis.
duct. endolymph. = ductus endolymphaticus.
d. reuniens = ductus reuniens Henseni.
endol. or endolymph. = appendix endolymphaticus.
rec. utr. = recessus utriculi.
sacc. = sacculus.
sac. endol. = saccus endolymphaticus.
sinus utr. lat. = sinus utriculi lateralis.
utric. = utriculus.
vestib. p. = vestibular pouch.



(N. VESTIBULARIS = RED N. COCHLEARIS = YELLOW)

(N. VESTIBULARIS = RED N. COCHLEARIS = YELLOW)

THE FINER STRUCTURE OF THE GLANDULA SUBMAXILLARIS OF THE RABBIT.

BY

BENSON A. COHOE.

From the Hull Laboratory of Anatomy, University of Chicago.

With 6 Figures.

Since the early researches of Boll, 69, the glandula submaxillaris of the rabbit has been the subject of many investigations on the part of histologists. This observer believed that the gland might well be regarded as the type of a whole important series of glands, formed on the same plan. The character of the cell, with its polygonal form, prominent nucleus and abundant cytoplasm, which after treatment with osmic acid became dark and granular, led him to consider it a pure serous gland. Later observers, notable among whom were Nussbaum, Langley, and Müller, were able to confirm his opinion as to the pure serous nature of the gland, and noted in addition that certain of the cells were loaded with granules, while others possessed a clear cytoplasm free from granulation. The weight of opinion was that these cells, unlike as to their cell contents, simply represented different physiological phases of one type of cell. The evidence presented so far to substantiate this view has not been altogether convincing, and in order to test the validity of such an opinion, a considerable number of glands, both in normal resting conditions and in various stages of activity has been studied during the present investigation in order to establish the identity or otherwise, the specificity of the different cells in this gland.

Historical.

The minute anatomy of the gl. submaxillaris of the rabbit was studied by Erik Müller, 96, in a series of investigation upon the relation of gland structure to physiological activity. He found that after sublimate fixation and staining with Heidenhain's iron hæmatoxylin, alternating transparent and darkly stained areas of gland tubules could readily be distinguished even with low magnification. By careful technique and

thin sections (2-4 micra), he was able to demonstrate certain structural differences in these two cell complexes. The cells of the darkly stained areas showed a cytoplasm filled with large, deeply stained granules. In some cases the granules filled the entire cell, while in other cells could be seen a lightly stained, fibrillar network, within the meshes of which the distinctly colored granules were held. The "transparent gland tubules" he found to consist of cells, the cell substance of which was formed of a fine network with thickened nodal points, forming regular clear spherical meshes. This second variety of cell did not fix so readily nor so well as the granule-holding cell and was frequently found shrunken and with irregular meshes, while at other times, under better conditions of fixation, the meshes appeared as only the expression of clear, round, unstained granules separated from one another by the colored network. Apart from cellular structure he believed that these two cell groups differed in no way from each other. "Only," he added, "it must be mentioned that the deeply stained tubules are found generally near an intercalated duct." To this rule, however, he claimed exceptions.

In order to explain the occurrence of these two varieties of cell forms, Müller considered two possibilities. Either the two forms of cells were of different kinds and yielded different secretions or they were identical and elaborated the same secretion, the apparent differences in structure resulting from different stages of activity. The second view appeared to him to be the correct one. There was not, he believed, a difference in kind between the cells but a difference in the state of function. Experimental evidence derived from an artificial stimulation of the gland, seemed to him to substantiate his opinion, when he found that after active secretion, the deeply colored areas of the gland parenchyma underwent a change as the granules disappeared. Here he was able to find transition stages between the light and dark cells. In case these granules might be simply artefacts in the sense of Fischer, he examined fresh tissue and found a similar and even more striking condition of differentiation between clear and dark cell areas, and furthermore that both kinds of cells contained granules, those of the dark areas being more highly refractive.

In the light of these observations, he formulated an hypothesis of the process of secretion of saliva in this gland. According to his view the cells of the clear areas contained the finished products of secretion. The unstained granules in these cells were transformed into the secretion vacuoles and these discharged their contents as saliva into the secretion

canaliculi, whence they passed into the lumen of the gland tubule. New granules were formed within the cell meshwork to replace those thus exhausted. These regenerated granules, lying within the meshes, at first small, increased in size and gave rise to the stainable granules of the dark cell areas. The latter, after attaining a certain size, lost their power of taking up stains and were transformed into the unstainable granules of the clear cells. In this manner arose the portions of the gland tubule containing clear cells.

Müller was not the first to observe that the cells of the submaxillary of the rabbit were wanting in homogeneity. Nussbaum, **77**, in an investigation of the process of ferment formation in glands chose this gland as the type of a pure serous salivary gland. After treatment with osmic acid he found that certain groups of cells assumed a deep black stain which distinguished them from other groups of cells within the gland. These dark granular cells he believed to be particularly rich in ferment.

Langley, **78**, also employed the submaxillary of the rabbit in his studies of ferment formation, and gave us valuable data concerning the secretory stages of this gland. He treated the gland with osmic acid, as did Nussbaum, but was unable to entirely confirm the results obtained by the latter observer. Nussbaum represented the intercalated ducts or ductules as composed of small, elongated cells, from which without intermediate forms there is a sudden change into the large cells of the alveolus. In this view he was supported by von Ebner, **72**. Langley found that, while it was true that certain cells forming the part of the alveolus immediately succeeding the ductule were more deeply stained than those of the more peripheral part of the alvolus, yet there was no section in which any marked difference in coloration between the cells lying next to the ductules and the cells of the ductules proper could be discerned. In all of his sections the intralobular ducts were stained most deeply, the ductules and neighboring cells less deeply, and the distal alveolar cells least of all. His conclusion was: "It appears to me, on the contrary, that the cells composing the ductule are but slightly elongated or not at all, and graduate as to size and appearance into the alveolar cells, so that of particular ones it is difficult to say whether they belong to a ductule or an alveolus, hence I name them transitional cells."

Held, **99**, in a series of researches upon granules and gland protoplasm studied the granule forms in the submaxillary of the rabbit. He distinguished three forms of cells characterized by three kinds of granules.

The first form of cell was filled with very highly refractive granules (" dunkle Zellen " of Erik Müller) ; another kind of cell contained very transparent granules, with a refractive index about equal to that of the protoplasm (" helle Zellen " of Müller). The third form of cell occurred least frequently and contained granules of low refractive power, the so-called " ring granules." Such forms were found in fresh sections of the gland taken from a rabbit after a fast of twenty-four hours. He employed various fixing reagents for studying stained sections. With alcoholic fixation he found that all three forms of granules had disappeared. With acetic acid the clear cells were destitute of granules, while the granules in the dark cells remained undissolved. After fixation in formalin and staining with Heidenhain's iron hæmatoxylin, he was able to distinguish two kinds of cells: (1) clear cells which contained only isolated granules; (2) cells, which in addition to granules, contained filaments comparable to the vegetative fibers of Altmann.

More recently Gerhardt, 03, has observed the granule complexes of this gland. He made a study of the changes in the salivary glands which followed upon section of the secretory nerves and found these granular areas in a gland sixteen weeks after section of the sympathetic in sections hardened in sublimate and stained with iron hæmatoxylin. He noted the occurrence of groups of granule-containing cells around the intercalated ducts but offered no explanation as to their nature beyond the suggestion of a possibility of their being artefacts.

Illing, 04, in an article upon the comparative anatomy of salivary glands regarded the submaxillary of the rabbit as a pure serous gland of the tubular variety. He noted a peculiar appearance in the structure of the gland in the fact that in each cell a border and a central zone could be distinguished: " In der Randzone erscheinen die Zellen vollgepfropft mit Körnchen, während nach dem Lumen hin die Körnchen sich mehr und mehr lichten und immer sparsamer werden, bis sie nahe dem Centrum schliesslich vollständig verschwunden sind."

Material-Technique.

In the preparation of a series of submaxillary glands, which might be regarded as representative of the different stages of physiological activity, pilocarpine was used to stimulate a flow of saliva. In each case the rabbit was deprived of food for a period of twenty-four hours prior to the administration of the drug. It was determined by experimentation upon several rabbits, that the greatest exhaustion of the gland appeared

at the end of from six to nine hours, with a dosage of 0.014 gms. per kilo of body weight of the animal. Profuse salivation commenced as a rule in from three to five minutes after the subcutaneous injection of this amount of pilocarpine, although certain of the animals manifested a marked idiosyncrasy towards the drug, as a result of which the stages of secretion as evidenced in sections of the gland revealed considerable variation at the end of the same number of hours, and with equivalent doses. In this manner a series of stages, taken at intervals of two hours was obtained, varying from the normal resting gland, through the phases of secretion, to a time twenty-four hours after the first administration of pilocarpine. At the end of this length of time it was found that the gland had again assumed a resting condition and that its microscopic appearance was that of a normal, unstimulated gland.

In each case fresh sections of the gland were examined in a medium of aqueous humor of the eye, which furnished a fluid which was at once isotonic and did not dissolve the granules within the cells. In addition to aqueous humor various reagents, referred to later, were used upon the fresh sections in order to study the conduct of the granules in different media. Small pieces of the remainder of the gland were hardened in different fixing reagents such as alcohol, formalin (10 per cent solution), Carnoy's fluid, aqueous sublimate, Kopsch's fluid, Zenker's fluid, and others.

The majority of these hardening fluids, with the exception of Kopsch's fluid, afforded little or no granular fixation. A modification of Kopsch's fluid, suggested by Dr. R. R. Bensley, consisting of a mixture of equal parts of Kopsch's fluid and saturated alcoholic sublimate, to which is added an equal volume of distilled water, gave better results than the simple Kopsch's fluid and served to fix the granules in a striking manner. A saturated solution of sublimate in normal salt solution gave good cellular fixation with scarcely any shrinkage, but did not preserve the granules. The most satisfactory results were obtained by the use of Bensley's alcoholic-bichromate-sublimate, which in addition to preservation of the granules, gave excellent cytoplasmic fixation. Even with this fluid, however, as with all of the reagents before mentioned, some shrinkage of the distal tubular cells was produced. Erik Müller, 96, has called attention to the difficulty in avoiding shrinkage in the fixation of these cells. After fixation the tissue was imbedded in paraffin and thin sections made varying from 2-5 micra in thickness.

For staining purposes Heidenhain's iron hæmatoxylin, followed by

eosin, erythrosin, or acid fuchsin and orange G. was found to give best results for the study of cytological structure. Various other special stains, referred to later, were employed for the differentiation of the cellular elements in the gland.

The Structure of the Normal Resting Gland.

If a thin, fresh section of the normal resting gland be examined in aqueous humor, with low magnification, the two cell groups described by Müller can readily be discerned. Many cells, occurring always in groups

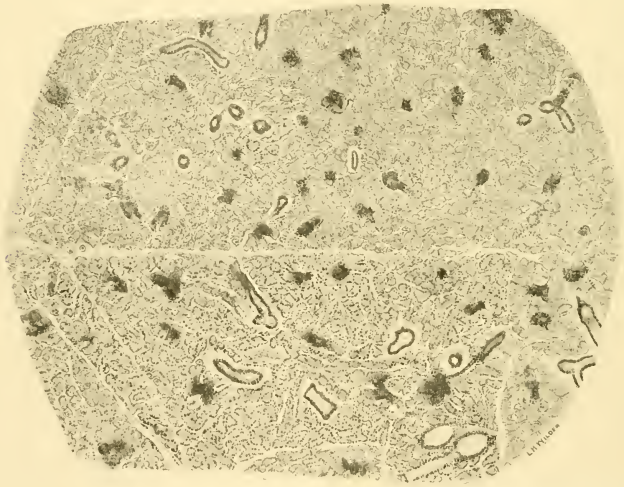


Fig. I. A section of the submaxillary gland of a rabbit after a fast of twenty-four hours. From a preparation, hardened in Bensley's alcoholic-bichromate-sublimate, and stained in hematoxylin and eosin. × 70.

are found to contain highly refractive granules, while other less conspicuous groups of cells are filled with granules of a low refractive index. Light and dark areas in the gland are thus mapped out with sharp distinctness, even with slight magnification. An examination under high magnification reveals the fact that all of the cells of both groups are loaded with granules. From this it can be at once inferred that the granules found in sections of the fixed gland are not artefacts produced by the precipitating action of the hardening fluid, but are in reality pre-existent in the cells. In the fresh preparation, a careful examination

discloses the fact that the cells of the dark groups occur invariably in close relation to the ductules. Repeated observations, both in fresh and hardened sections, fails to show any exception to this rule.

The granules seen in the dark cells in a fresh section appear to be smaller in size than those in the light cells. Considerable variation is to be noticed in the size of the individual granules in the dark cells. The nuclei in both kinds of cells, in a resting gland, are obscured by the great abundance of granules. The action of certain reagents on these unfixed granules gives striking evidence of the ready solubility of the granules in both kinds of cells. Those found in the clear cells are especially soluble in most reagents. With isotonic salt solution the granules are not preserved but slowly dissolve. If placed in a one per cent solution of acetic acid, the section at once becomes opaque and the granules cease to be spherical, although remaining visible, while the two cell groups can be no longer distinguished. Glycerine, in concentrated solution, also dissolves the granules. After treatment with osmic acid (1 per cent solution), the lumina of the ducts are seen to be crowded with dark brown granules, while the granules in the dark cells assume a brownish black coloration. When treated with alcohol, or with aqueous sublimate, the granules are readily dissolved. Solger, 96, moreover, has called attention to their ready solubility in dilute solutions of chromic acid, potassium bichromate, acetic acid, and pure water.

In a thin section (2-3 micra) of a resting gland, fixed in Bensley's fluid, and stained with hæmatoxylin and eosin, there can be readily observed, even with low magnification, in the portion of the gland near an intercalated duct, a group of cells loaded with granules, which stain intensely with eosin. (Fig. I.) These centrally disposed cells are followed by a second group of cells, the cytoplasm of which stains indifferently with eosin and is destitute of granules. At no time does this second variety of cell lie in close relation to an intercalated duct, while the granule-holding cell, upon repeated observations, is found always opening directly into a ductule. After staining with Heidenhain's iron hæmatoxylin the granules assume a deep blue-black color and are seen to be crowded towards the lumen of the tubule. The nuclei, when visible among the mass of granules, appear oval in form and are situated towards the base of the cell. This proximally disposed group of granular cells is of constant occurrence throughout the lobule and has a variable diameter of from 85-200 micra. The individual cells of the group have an average diameter of 24 micra, while the diameter of the nucleus averages about 11 micra.

The clear cells of the distal group present a well-marked cytoplasmic net-work, forming large meshes. (Fig. II.) The cytoplasm stains feebly with most of the ordinary stains. The nuclei of these cells are

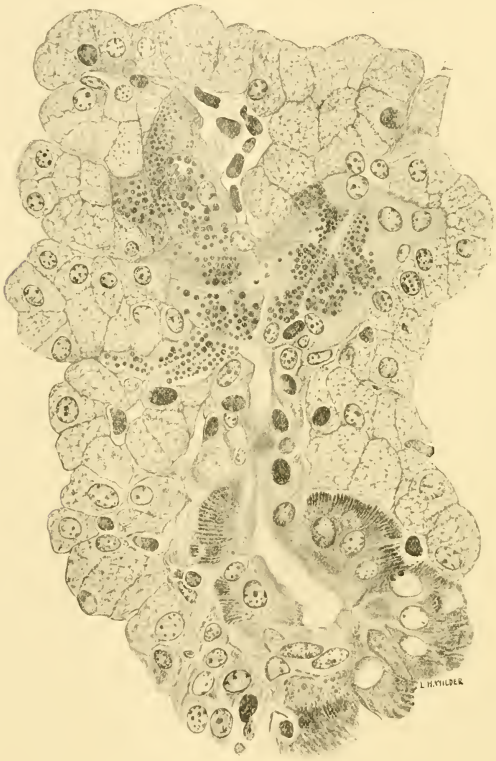


Fig. II. A portion of the same gland shown in Fig. I more highly magnified. Preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

on the whole smaller and less spherical than those of the granule cells, and are located nearer to the base of the cell than in the other variety of cell. These cells, even after the shrinkage with which their fixation is always attended, possess a somewhat larger diameter than the granule

cell. Their average diameter is about 28 micra, while that of the nucleus is 9 micra. Secretory canaliculi occur abundantly in connection with these outer cells, and are seen to ramify between the cells. (Fig. III.)

The evidence adduced by a study of sections of the normal resting gland, stained with certain selective dyes, is most convincing of the specificity of these two kind of cells. Sections hardened in Bensley's fluid, and stained with iron hæmatoxylin and orange G., show the cytoplasm of the granule-holding cells deeply stained by the orange G., while the cytoplasm of the clear cells takes on a bluish tint from the hæmatoxylin. The granules stain a deep blue-black. With Bensley's neutral gentian method the granules are colored an intense violet. A sharp differentiation between the two kinds of cells is afforded by means of Mann's methyl-blue eosin, the granules and cytoplasm, of the granular

Fig. III. A section through a group of distal alveolar cells, showing secretion canaliculi. From a preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

and duct cells staining a deep red, while the cytoplasm of the clear cells is colored by the methyl blue. With Nissl's methylene blue the intralobular duct and granular cells stain metachromatically, a reddish violet, and the clear cells are stained blue. Dahlia (1 per cent aqueous solution) also stains the cytoplasm of the granular cells metachromatically; the granules assume a decided reddish color. With this stain the clear cells and nuclei are blue, and the cytoplasm of the intralobular duct cells takes on a violet color. By means of iron hæmatoxylin and erythrosin, a clear differentiation can be established. The cytoplasm of the granular cells stains a deep pink in contrast with the black granules, while the cytoplasm of the clear cells is stained a feeble pink by the erythrosin. Orcein stains both kinds of cells, the cytoplasm of the intralobular duct cells taking up the stain intensely, while the granular cells also stain strongly; the cytoplasm of the clear cells displays much less affinity for

the stain. Unna's polychrome methylene blue stains the clear cells blue and leaves the granular and duct cells with scarcely any coloration.

In sections stained with hæmatoxylin and Congo red, the cytoplasm of the duct and granular cells is strongly colored by the Congo red, while the clear cells take up the blue of the hæmatoxylin. By means of the Ehrlich-Biondi stain, the intralobular duct cells are dyed an orange-yellow, the granules and cytoplasm of the granular cells an intense red, and the clear cells a violet-blue. This stain was employed by Krause, 95, in his study of the glandula submaxillaris of the hedgehog (erinaceus europæus) and the results obtained bore a striking similarity to the conditions found in the same gland in the rabbit with the use of the same stain. With toluidine blue the granular cells and granules stain a bluish-green, while the clear cells take on a fairly strong blue color. After fixation in Kopsch's fluid, and staining with a mixture of lichtgrün and safranin, the clear cells and all of the nuclei take up the brilliant red of the safranin, and the granular cells, as well as the intralobular duct cells, assume a violet blue. With Ehrlich's neutral solution the granules stain an intense red, as does also the "rodded" cytoplasm of the cells of the intralobular ducts; the cytoplasm of the clear cells stains blue.

By the use of the so-called specific mucin stain of Krause, thionin, followed by potassium ferrocyanide, the clear cells stain metachromatically, giving the red color characteristic for mucous cells. The granular and duct cells stain with the thionin and remain unchanged after treatment with the potassium ferrocyanide. A similar and suggestive result may be obtained by staining sections hardened in Kopsch's fluid with Mayer's muchæmatein, as modified by Bensley, 03. The clear cells when thus treated give a well-marked "mucous" reaction, while the granule and duct cells remain unstained. With other ordinary methods of fixation, this so-called mucous reaction cannot be obtained in so striking a manner. After formalin fixation, however, the clear cells give a feeble reaction with muchæmatein.

In order to study the relation of the cells of the granular areas to the intercalated ducts, a number of Golgi preparations of the gland were made. With the resulting impregnation, the granular cell complexes appeared as dark brown masses, while the clear cells were stained a light yellow. The injected secretion canaliculi were found present in both varieties of cells. In the cells of the granular areas the canals appeared short, broad and unbranched, a condition which can readily be observed in sections of the gland, hardened in aqueous sublimate, and stained with

iron hæmatoxylin. In these Golgi preparations the cells of the granular area were found invariably lying next to an intercalated duct. They, in turn, were constantly succeeded by a group of clear, non-granular cells, occupying a distal position in the tubule.

The intralobular ducts of this gland are lined by a single layer of epithelial cells of a high columnar type. This layer rests upon the basement membrane, outside of which there is a scanty amount of connective tissue. The nuclei of the cells are large and spherical and occupy a position central in the cell. A well-marked nucleolus is present, but the chromatin is less abundant than in the nuclei of the gland cells. The cytoplasm is abundant and stains readily with acid dyes. Towards the base of the cells it is arranged in the form of longitudinal striations,

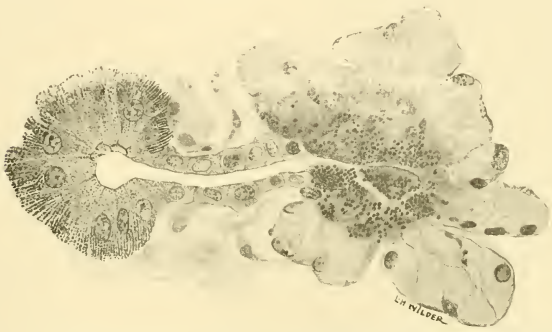


Fig. IV. A gland alveolus, with its accompanying intercalated duct leading into an intralobular duct. Preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

giving rise to the "rodded" variety of cell constituting the "salivary tubes," described by Pflüger, who believed that this form of cell was actively concerned in the secretory processes of the gland. In an exhausted gland, after prolonged stimulation, the cytoplasm is observed to stain more feebly than in the resting gland. Large secretion granules are never found in these cells, but the cytoplasm itself manifests a finely granular appearance.

The ductules, or intercalary ducts, open into the intralobular ducts, obliquely or at right angles, with an abrupt change from the low, flat cubical cell of the ductule to the high columnar, rodded cell of the intralobular duct. (Fig. IV.) The cells are elongated in form, contain but little protoplasm and are devoid of granules. The reaction of the

cytoplasm towards stains is similar to that of the cells of the ducts and of the granular areas. The nuclei are oval and are situated towards the central part of the cell. The change from the elongated, non-granular ductule cell to that of the large cubical, granule-holding cell of the granular area is also an abrupt one. Langley, 78, working from a physiological point of view, believed that there was a gradual transition from the cells of the ductule to the cells of the alveolus. He treated sections with osmic acid, which affords at best a poor protoplasmic stain. Nussbaum, 77, represented the ductules as formed of small, elongated cells, from which without intermediate forms, there is a sudden change to the large alveolar cell. By the use of osmic acid he found them to contain granules. Von Ebner, 72, had previously supported the view of a sudden change from the low, cubical cell of the ductule into the large alveolar cell. Lastly Klein, 82, has noted that the flattened, elongated, epithelial cells of the ductules pass directly into the columnar secreting cells of the large alveoli. An examination of preparations of thin sections, stained with iron hæmatoxylin, confirms the correctness of the observations of Nussbaum, von Ebner, and Klein. One hesitates to dispute an opinion, entertained by so distinguished an observer as Langley, but his technique was for physiological, not cytological purposes. In a later paper, 79, his opinion in regard to a graduation in form and size of cell was not so positive; he says, "Some of the cells, which, from their shape, certainly would be called ductule cells, certainly contain granules, though they are, I am inclined to believe, absent from the ductule cells, springing immediately from the ducts. The absence of cell outlines and the difficulty of obtaining thin sections of the fresh gland, makes a decision on this point difficult to arrive at."

The question of the existence of a differentiated, tubular portion of the gl. submaxillaris, designated by the name of Bermann's gland, has been the subject of much controversy among investigators of salivary glands. Bermann, 78, described in connection with the submaxillary gland of the rabbit, a small tubular portion of the gland enclosed in the same connective capsule. This part of the gland he believed to be an "organ sui generis." In the new born rabbit he found it to be very small and lying in close proximity to the hilus of the gland. According to him a similar tubular portion occurs in the submaxillary glands of the guinea pig, bat, dog, cat, and fox.

A number of other authors have confirmed, in the main, the observations of Bermann. Langley, 79, found that sections of the gl. submaxillaris of the rabbit, made near the entry of the ductus Whartonianus.

usually displayed a lobule or a portion of a lobule, consisting of branching tubules in place of the ordinary alveoli or ducts. W. Krause, 84, believed that this gland was undoubtedly a morphologically and functionally differentiated portion of the submaxillary gland, tubular in form, which represented a rudiment of the gl. sublingualis, the connection of which with the ductus submaxillaris was altogether secondary.

The mass of evidence, brought by the majority of investigators, is opposed to the existence of a clearly differentiated portion of this gland in the sense of Bermann. Beyer, 79, after a series of researches upon this subject, maintained that Bermann's gland was none other than a gl. sublingualis. Von Ebner, 99, as a result of experimentation, regarded Bermann's gland as being produced by a stasis of secretion in the broad lumina of the gland tubules. Illing, 04, after a comparative study of the gl. submaxillares of the domestic animals, could not confirm the presence of this gland: "Eine sog. Bermannsche Drüse, wie sie von Bermann als eine deutlich differenzierte, besondere, zusammengesetzte tubulöse Drüse in der submaxillaris beschrieben wurde, habe ich weder bei Hunde und Katze noch beim Kaninchen oder irgend einem anderen Tiere konstatieren können." In order to substantiate his opinion, he sectioned the entire submaxillary glands of several rabbits, and reconstructed models of the same. In no case did he find a differentiated tubular portion of the gland. He concurs with the view, expressed by a number of other observers, that Bermann's gland is simply a portion of the gl. sublingualis (polystomatica), which frequently lies in such close proximity to the gl. submaxillaris as to be sectioned along with it. S. Mayer, 94, in his Gland Studies, has noted that forms conforming with Bermann's description ("rein tubulösen Drüse") may be found in the gl. submaxillaris and parotis of the rabbit, and in the gl. parotis of the dog. Among the structures occurring in the region of the submaxillary gland, presenting sources of errors for observers, he mentions the "Winterschalfdrüse," found in many animals in this region. In the female rat he has noted the presence of mammary gland tissue extending into the submaxillary region, and he suggests the possibility of Bermann having mistaken degenerated mammary gland tissue for a differentiated portion of the submaxillary: "Es ist leicht möglich, dass Bermann in den von ihn geschilderten, in der Nachbarschaft der Submaxillardrüse gelegen 'rein tubulosen Drüsen,' auch rückgebildete Milchdrüsen läppchen vor sich hat."

In a study of many glands during the present investigation a specially differentiated part of the gland, as described by Bermann, was in no instance observed.

The Stages of Physiological Activity.

The microscopic appearances of the hardened and stained sections of the gland, taken at any stage of activity, simulated closely the conditions found in the preparation of the fresh gland of the corresponding stage, with this notable exception, that while in fresh sections of the gland, granules were found present in both varieties of cells, in the fixed sections they occurred only in the group of cells lying next to an intercalated duct. No reagent so far employed has had the property of fixing, to any extent, the granules in the clear distal group of cells. Always associated with this lack of granule fixation, there was present more or less shrinkage of the cell cytoplasm. It was, moreover, to be observed, that the newly formed granules, in a gland recovering from exhaustion, were more readily soluble than the granules of a resting gland, for which reason, while in the fresh tissue abundant granules might be present, the corresponding fixed specimen might display scanty granulation.

After a period of three hours' stimulation with pilocarpine a thin, fresh section of the gland, examined in aqueous humor, showed all of the cells still loaded with granules. In the cells of the dark areas, close to the ductules, the granules varied much in size and occurred in irregular masses. The cells of the clear areas were still filled with granules. The nuclei were discernible with difficulty. In a fixed preparation of the gland, stained with Heidenhain's iron hæmatoxylin and eosin, the cells were seen to be in a condition of partial exhaustion. There were fewer granules present in the cells of the dark areas and the cytoplasm of these cells was clearly differentiated from the more lightly stained cytoplasm of the cells of the clear areas. With eosin the cytoplasm of the granular cells was stained a lively red, while that of the distal, clear cells was feebly colored by this stain. The nuclei of the granular cells were now more readily visible than in the resting gland, and showed a tendency to assume a position more central in the cell. Secretion canaliculi could now be seen in both kinds of cells, but were observed to be more numerous in the clear cells. In the granule-holding cell they were found to be short and broad without any apparent branching.

At the end of four hours in fresh sections, the blood vessels of the gland were seen to be much congested, and both cell complexes showed fewer granules. It was to be noted that the granules in the cells of the dark areas appeared to persist longer under stimulation than those in the cells of the clear areas. After seven hours the gland was found shrunken, with the blood vessels engorged. The two cell complexes could

be differentiated only with difficulty, while all of the cells appeared exhausted and almost destitute of granules. In the cells of the dark areas some few small granules were still present, and in some cells border fat granules could be seen. The gland tubules appeared shrunken while the nuclei were readily visible and were found to be round and small. The hardened and stained specimens of the gland taken from these stages revealed conditions which corresponded closely to those of the fresh sections.

The stage of most complete exhaustion was obtained from an animal killed nine hours after stimulation. The gland here appeared pale

Fig. V. A section of an exhausted gland of a rabbit, after a fast of twenty-four hours and subsequent stimulation with pilocarpine for a period of nine hours. From a preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

and shrunken. In fresh sections, examined in aqueous humor, the cells were found to be free from granules, with the exception of the dark cells in which a few still persisted. At no time did the granules entirely disappear from these cells, a fact that accords with the observations of Langley, 78. The few granules remaining in the dark cells were irregular in form and of unequal size. The cells bore evidence of shrinkage and the nuclei had lost their rotundity. In a fixed and stained specimen of this stage (Fig. V), the distinction between the two kinds of cells was less characteristic than in the resting gland. The clear cells appeared even

more shrunken than in the normal gland. The protoplasmic network was formed of larger and more irregular meshes, which did not stain so readily as in the resting gland. The nucleus was observed to be large with no apparent shrinkage, and with nuclear membrane and nucleolus well marked. The chromatin, on the other hand, was not so abundant. The location of the nucleus was more central in the cell and further removed from the basement membrane, than in the resting gland, while its form was more spherical with less apparent distortion.

The cells of the granular areas in the exhausted gland were recognized with greater difficulty in stained sections on account of the loss of secretion granules through active stimulation. Even here, however, a few granules still persisted. Those remaining were smaller than the granules found in a normal gland and were found wholly in the distal part of the cell, lying in close proximity to the lumen of the tubule. The cytoplasm was diminished in amount and revealed a reticular meshwork. It stained feebly in marked contrast to the strongly staining character of the cytoplasm of the resting granular cell. The nuclei were located towards the center of the cell and showed little evidence of shrinkage. They were observed in this stage, as in the resting gland, to be slightly larger than the nuclei of the clear cells, and to be poor in chromatin.

A section of the gland in the exhausted stage, stained with iron hæmatoxylin, afforded a clear view of the secretion canaliculi. The lumen of such a canal, as well as the lumen of a tubule, appeared broader than in the resting gland. Ramifications of the canaliculi could plainly be observed among the clear cells but were absent from the granular cells. The cells of the intercalated and intralobular ducts were somewhat shrunken and fallen away from the basement membrane; the protoplasmic striations of the "rodded" duct cells were found to stain less intensely than in the resting gland. The blood-vessel in the interlobular connective tissue were engorged with red corpuscles.

The next stage was taken from a gland twelve hours after stimulation, when both kinds of cells were found to again contain granules in a fresh section. In the cells of the dark areas many granules were present, while the clear cells were seen to contain fewer and smaller granules. It was, moreover, quite obvious that the dark cell groups occurred in close relation to the ductules, in the same position as in the resting gland. After a period of twenty hours, the gland, although somewhat shrunken, had again assumed in a large degree, the appearance of a normal resting

gland. In fresh sections the cells and nuclei were found to be but little shrunken. Both areas could be distinguished but not so readily as in the normal unstimulated gland. The tubules surrounding the ductules contained many granules, none of which were of great size. In a hardened and stained section of this stage, the cells of the dark areas were found to again contain many granules, the nuclei being for the most part obscured. The clear cells were now less shrunken, and the cyto-

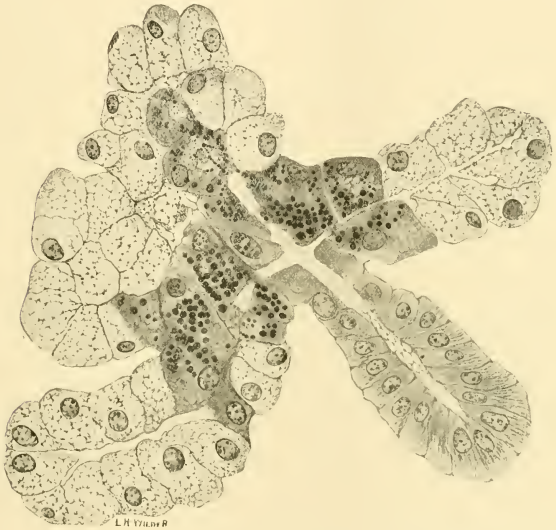


FIG. VI. A section of the gland of a rabbit killed twenty-four hours after stimulation with pilocarpine. Preparation stained in iron-alum hematoxylin. Leitz Homog. Imm. 1/12. Oc. 4.

plasm stained more vigorously. The nuclei gave the appearance of again being displaced towards the base of the cell by the abundant formation of secretion, and were more or less irregular in contour. It was, moreover, to be noticed in stained, just as in fresh preparations, of this gland, returning to a normal resting condition, that the granule cell-complex was again intermediate in position and situated, in every case, in close relation to an intercalated duct.

The final stage taken for the study of secretion phases, was from a

gland stimulated twenty-four hours previously. Here the appearance was found to be that of a normal resting gland. In fresh sections all of the cells were found loaded with granules. The dark cell areas could be readily detected, and were seen to occur regularly around the ductules, just as in the unstimulated resting gland. The cells of these areas were filled with granules, much larger than those in the cells of the clear areas. These latter cells were loaded with small granules of low refractive power, and while some of the cells were completely filled, in others the granules were found only towards the lumen of the tubule. The nuclei in most of the cells were not visible, owing to the mass of granules. When distinguishable, they were found close to the base of the cell, and were spherical in form. There was no evidence in the gland of degenerative processes. In a fixed and stained specimen, taken from this stage (Fig. VI), there could be observed little or no deviation from the appearance of a normal resting gland. The cells of the dark areas, occurring around the ductules, were found loaded with granules, while the granules in the cells of the clear areas remained unfixed, just as in the normal gland.

Discussion.

After an examination of the different physiological phases of the gl. submaxillaris of the rabbit, conducted in the manner described, and a study of the morphology and microchemical reactions of the cells of the gland, one is forced to abandon the view held by some earlier observers, that it is a pure serous gland formed of cells of one and the same type. The following observed facts justify us in regarding it as a composite gland with at least two kinds of cells in the secreting tubules, and furthermore, that these two varieties of cells do not represent different secretion phases of the same cell but are morphologically and physiologically distinct:

(1). In fresh sections of the gland, two distinct areas can be observed, each composed of a group of cells containing granules with a refractive index different from that of the granules in the cells of the other group, indicating a difference in chemical composition. The granules in the cells of the two groups behave in a different manner towards various reagents.

(2). The cells forming the "dark" areas in hardened sections always contain granules and are always associated in close relation with an intercalated duct. In such fixed and stained specimens the distal group of cells, composing the "clear" areas are always destitute of granules.

(3). An examination of the secretion phases, observed in fresh sections, reveals the fact that under stimulation, the granules of the clear cells disappear earlier than those of the dark cells, while in a gland recovering from exhaustion, those in the clear cells are the later to reappear.

(4). In stained preparations of stimulated glands, the granules are found to have disappeared from the cells of the dark proximal group, and to have reappeared in a gland recovering from exhaustion, in one and the same region, around an intercalated duct. The cells of the distal clear group, in a section prepared after fixation in any of the fluids employed, are never found to contain stainable granules during any phase of secretion.

(5). The cytoplasm of the two kinds of cells exhibits different staining properties and affords distinctly different microchemical reactions. The proximally disposed granule-holding cell conforms to the classic description of a serous cell, while the clear distal cell resembles in some degree the appearance of a mucous cell.

The significance of the presence of these two types of cells in this gland is as yet doubtful, and must remain so until our knowledge of the chemistry of the submaxillary saliva of the rabbit is more exact and the microchemical methods at our disposal further developed. The rôle of each cell in the production of the secretion is unknown and, indeed, even the presence of a diastatic ferment in this saliva is still a debated question. Many suggestive, but by no means convincing, results were obtained concerning the nature of these cells through their staining and microchemical properties.

The cytoplasm of the cells afforded many valuable criteria for differentiating the two types. In the clear cells it is less abundant than in the granular cells and contains a basophilic recticulum. It possesses a substance which has a marked affinity for hæmatoxylin and basic stains. With acid stains it does not stain so readily as the cytoplasm of the granular cell. With P. Mayer's muchæmatein, as modified by Bensley, **03,** in sections fixed with Kopsch's fluid, a typical "mucous" reaction was obtained in these clear cells. With Krause's so-called mucin stain (thionin followed by potassium ferrocyanide) these cells also behaved as mucous cells and gave a metachromatic red color. Neither of these stains, however, can be regarded as specific for mucin. According to Hoyer, **90,** a metachromatic color with thionin is a positive indication of mucin or a mucin-like body. Krause, **95,** tested the validity of this assertion by successfully staining sections of hyaline cartilage and of

the epithelium of the gall bladder, and concluded that the reaction is not specific for mucin. Although such reactions, along with the accompanying morphological structure of a large, clear cell, of indifferent staining power, and a somewhat flattened nucleus lying in the proximal zone of the cell, is suggestive of the mucous nature of these distal tubular cells of the rabbit's submaxillary, we are not warranted in interpreting them as such, at least in the light of our present meager knowledge of the chemistry of the mucins. The researches of Langley, 86, and Hammarsten, 85, have shown that the elaboration of mucins by the secreting cells is a process involving several stages. Mayer, 97, constructed a graded series of mucins, ranging from those which stained with difficulty in muchæmatein to mucin which stain readily and deeply with the same dye. Conversely he was able to obtain a typical mucin reaction in the cells of the gl. submaxillaris of the hedgehog, a gland which, according to Krause, does not secrete mucin. Bensley, 02, in a study of the cardiac glands of mammals has called attention to the fact that it is unreasonable to expect that the different mucins and different stages of the elaboration of mucin would present always the same staining properties. It is possible that future researches may prove that these cells secrete a mucin-like substance.

The second type of cell is found in the group of granule-containing cells in the region of an intercalated duct and presents the well-known picture of a serous cell. The cytoplasm is abundant and stains readily and deeply with ordinary dyes. The nucleus is prominent, lies towards the center of the cell, and is spherical in form. If a ferment were elaborated by this gland, it would be natural to conclude that the cells of this second type gave rise to it, and that the granules found within them were zymogenic in character. From the fact that the granules stained black with osmic acid Nussbaum, 77, concluded that these cells were particularly rich in an amylolytic ferment and that the distal tubular cells were not concerned in its formation. Langley, 78, and Grützner, 78, were unable to confirm his observations and believed that an amylolytic ferment was absent from this gland. With Macallum's, 95, microchemical test for iron, a negative result was obtained. The presence of iron either in the granules or in the cytoplasm could not be demonstrated. The nitro-molybdate reaction, employed by Macallum, 98, to detect phosphorus microchemically in the tissues, also gave a negative result. Prozymogen, either in a diffused form in the proximal zone of the cell, or irregularly distributed in the basal cytoplasm in the form of "basal filaments" of Solger was accordingly regarded as absent.

A comparison of the cellular structure of this gland with the gl. submaxillaris of the hedgehog (*Erinaceus europæus*) discloses a similarity of structure which is at once both striking and suggestive. This gland in the hedgehog was first described by Kultschizky, **85**, and later by Krause, **95**. In the tubules of the gland are found granular and non-granular cells. With Biondi's stain the granules assume an intensely red color, as does also the meshwork found within these cells. The granule-containing cell in the rabbit's submaxillary react similarly with Biondi's stain. The cells destitute of granules, which occur in the peripheral part of the tubule in the hedgehog's gland are stained blue, while the clear, non-granular cells of the rabbit's submaxillary are colored a violet-blue with this stain. The cytoplasm of these outer peripheral cells possesses a well-marked network, within which granules are never found. The nucleus is often irregular and lies close to the basement membrane. Kultschizky described the granular cells as serous, and the cells lying outside of the latter and not containing granules as mucinoid. He was able to show that the two kinds of cells in this gland were arranged in regular groups and that the serous cells did not appear in the form of demilunes, as in the mixed salivary glands of other animals but occupied once in a while a great part of the gland lumen.

Krause also called attention to the similarity existing between the submaxillary glands of the rabbit and hedgehog: "Es lag natürlich sehr nahe der Submaxillaris des Kaninchens, in welchem wie Nussbaum zuerst beschrieben hat, die Endstücke der Ausführungsgänge zahlreich sich in Osmium säure intensiv schwärzende Granula enthalten, welche der erwähnte Autor für Ferment Körnchen halt."

The relation of the two kinds of cells to each other in this gland of the hedgehog, while quite simple does not display the constancy nor regularity of position found in the corresponding gland of the rabbit, where the granule-containing cells always occupy a position intermediate between an intercalated duct and the group of clear or non-granular cells. In the hedgehog the tubule of granular cells branches simply, and its end piece is covered with these mucinoid cells. The latter are related in such a way to the former that mostly the middle of a small lobule contains only granular cells, which are surrounded by a garland of non-granular cells. This relationship Krause verified by means of Berlin blue injection.

The similarity in the behavior of these cells of the two glands towards stains and in their microchemical reactions is very marked. In both

glands the granule-containing cells are oxyphilic and the non-granular basophilic. When fresh sections are placed in a one per cent solution of pyrogallic acid in both glands the rodded epithelium of the ducts and the granule-containing cells are stained a lively brown, while the second kind of cell manifests no reaction. Merkel, 83, employed pyrogallic acid to demonstrate the presence of calcium salts microchemically in the salivary glands. Hence Krause concluded that the granule-containing cells secrete the bulk of the lime salts and albumin of the submaxillary saliva of the hedgehog. On the other hand sections of the rabbit's submaxillary when treated with a solution of ammonium purpurate for the detection of calcium salts failed to show the presence of calcium either in the cells of the ducts or of the granular areas. A brown coloration with pyrogallic acid cannot be regarded as a test for the presence of calcium.

That both glands when treated with Krause's mucin stain give a similar reaction has been already mentioned in the present paper. In the hedgehog the granular (serous) cells stain a weak blue and the non-granular mucinoid cells take on the simple metachromatic red color characteristic of mucin. The submaxillary of the rabbit when treated with this stain gave a similar result; the granular cells stained blue and the clear cells a metachromatic red. Although this stain, as before pointed out, is not specific for mucin, it nevertheless serves to indicate a similarity in the chemical composition of the cytoplasm in the corresponding cells of these two glands.

In concluding it is a pleasure to thank Professor R. R. Bensley for the many valuable suggestions offered during the preparation of the present paper.

LITERATURE CITED.

Bensley, R. R., 02.—The Cardiac Glands of Mammals. Am. Journ. Anat., Baltimore, Vol. II, No. 1, pp. 105-156, 1902.

——— 03.—The Structure of the Glands of Brunner. The Decennial Publications of the University of Chicago, Vol. X, Chicago, 1903.

Bermann, I., 78.—Ueber die Zusammensetzung der Glandula submaxillaris aus verschiedenen Drüsenformen und deren funktionelle Strukturveränderungen. Würzburg, 1878.

Beyer, G., 79.—Die Glandula sublingualis, ihr histologischer Bau und ihre funktionellen Veränderungen. Inaug. Diss., Breslau, 1879.

Boll, F., 69.—Die Bindesubstanz der Drüsen. Arch. f. mikr. Anat., Bonn, Bd. 5, S. 334-355, 1869.

Ebner, von V., 72.—Ueber die Anfänge der Speichelgänge in den Alveolen der Speicheldrüsen. Arch. f. mikr. Anat., Bonn, Bd. 8. S. 481-513, 1872.

——— 99.—Koelliker's Handbuch der Gewebelehre des Menschen, Bd. 3, Leipzig, 1899.

Gerhardt, U., 03.—Ueber die histolcgische Veränderungen in den Speicheldrüsen nach Durchschneidung der secretorischen Nerven. Arch. f. d. gesammte Physiol. Bd. 97, S. 315-334, 1903.

Grützner, P., 78.—Ueber Bildung und Ausscheidung von Fermenten. Arch. f. d. gesammte Physiol., Bd. 16, S. 105-123, 1878.

Hammarsten, O., 85.—Studien über Mucin und mucinahnliche Substanzen. Arch. f. d. gesammte Physiol., Bonn, Bd., 36, S. 373-456, 1885.

Held, H., 99.—Beobachtungen am tierischen Protoplasma. I. Drüsengranula und Drüsenprotoplasma. Arch. f. Anat. u. Physiol., Anat. Abt., S. 284-312, 1899.

Hoyer, 90.—Ueber den Nachweis des Mucins in Geweben mittelst der Färbemethode. Arch. f. mikr. Anat., Bonn, Bd. 36, S. 310-374, 1890.

Illing, G., 04.—Vergleichende makroskopische und mikroskopische über die Submaxillaren Speicheldrüsen der Haussaugetiere. Anatomische Hefte, Bd. 26, 1904.

Klein, E., 82.—On the lymphatic system and the minute anatomy of the salivary glands and pancreas. Quart. Journ. Microsc. So., Vol. XXII, pp. 154-175, 1882.

Krause, R., 95.—Zur Histologie der Speicheldrüsen. Die Speicheldrüsen des Igels. Arch. f. mikr. Anat., Bd. 45, S. 93-133, 1895.

Krause, W., 84.—Die Anatomie des Kaninchens. 2. Aufl., Leipzig, 1884.

Kultschizky, N., 85.—Zur Lehre vom feineren Bau der Speicheldrüsen. Zeitschr. f. wiss. Zool., Bd. 41, S. 99-106, 1885.

Langley, J. N., 78.—Some remarks on the formation of ferment in the submaxillary gland of the rabbit. The Journ. of Physiol., Vol. I, pp. 68-71, 1878.

——— 79.—On the structure of serous glands in rest and activity. Proc. of the Royal Soc., London, Vol. 29, pp. 377-382, 1879.

——— 84.—On the structure of secretory cells and on the changes which take place in them during secretion. Internat. Monatschr. f. Anat. u. Histol., Leipzig, Bd. I, S. 69-75, 1884.

——— 86.—On the structure of mucous salivary glands. Proc. of the Royal Soc., London, Vol. XL, pp. 362-367, 1886.

Macallum, A. B., 95.—On the Distribution of Assimilated Compounds of Iron other than Hæmoglobin and Hæmatins in Animal and Vegetable Cells. Quart. Journ. Micr. Sc., London, Vol. XXXVIII, pp. 175-274, 1895.

——— 98.—On the Detection and Localization of Phosphorus in Animal and Vegetable Tissues. Proc. Roy. Soc., London, LXIII, pp. 468-479, 1898.

Mayer, P., 97.—Ueber Schleimfärbung. Mitteil. d. zool. Station zu Neapel, Leipzig, Bd. 12, S. 303-330, 1897.

Mayer, S., 94.—Adenologische Mitteilungen. Anat. Anz., Bd. 10, No. 6, S. 177-191, 1894.

Merkel, F., 83.—Die Speichelröhren. Rektoratsprogramm, Leipzig, 1883.

Müller, E., 96.—Drüsenstudien. I. Die serösen Speicheldrüsen. Arch. f. Anat. u. Physiol., Anat. Abt., H. 5-6, S. 305-323, 1896.

Nussbaum, M., 77.—Ueber den Bau und die Thatigkeit der Drüsen. I. Mitteil. die Fermentbildung in den Drüsen. Arch. f. mikr. Anat., Bd. 13, S. 721-755, 1877.

Solger, B., 96.—Ueber die feineren Bau der Glandula submaxillaris des Menschen mit besonderer Berücksichtung der Drüsengranula. Festschr. z. 70. Geburtstag von Carl Gegenbauer, Bd. 2, S. 179-248, 1896.

THE RELATION BETWEEN THE CYTO-RETICULUM AND THE FIBRIL BUNDLES IN THE HEART MUSCLE CELL OF THE CHICK.

BY

HARRY LEWIS WIEMAN.

From the Biological Laboratory of the University of Cincinnati.

WITH 2 DIAGRAMS AND 17 FIGURES.

In the great mass of literature that has appeared on the subject of the striated muscle, no account apparently exists of the histogenesis of the heart muscle cell of the chick. This seems rather strange in face of the fact that the chick always has been the classic subject for embryological research. The present study was undertaken for the purpose of determining the structures existing in the heart muscle cell of the chick, especially for comparison with results of similar work that has already been done on other vertebrate forms.

The particular phase of the histogenesis treated in this paper is the relation between the cyto-reticulum of the embryonic cell and the fibril bundles as found in the adult muscle tissue. To avoid any possible confusion or misunderstanding, a definition of terms here at the outset will perhaps not be amiss.

By cyto-reticulum, the writer means the deeply staining network found traversing the cytoplasm of early embryonic cells. The fibril bundles, corresponding to the " Muskelsäulchen " of Koelliker, 02, are the striated longitudinally disposed masses running the length of the adult cell. Each fibril bundle is composed of more elementary parts called fibrils.

On account of the abundance of material and the ease with which the various stages in the development of the cell can be secured, the chick is very well adapted to a study of this kind. The one serious objection is that the various structures of the cell are not as well differentiated as in some other forms.

To Professor Guyer, at whose suggestion this work was taken up, the writer is much indebted for valuable assistance.

Methods.

A detailed study of the cytoplasmic structures of the heart muscle cell requires the use of a very high magnification. To secure good definition, it was found necessary to make sections 3 μ in thickness. Sections of 4 to 10 μ thickness were used for general structure and form of the cell. Sections were cut in paraffin.

Of the different fixing agents used, none gave more satisfactory preparations than Kolossow's, 92, solution. None was found to surpass it in faithful preservation and differentiation of the cytoplasmic structures. The tissue is killed and hardened by treatment for 15 minutes with a 1 per cent osmic acid solution. This is followed by immersion for the same length of time in a reducing mixture of pyrogallol and tannin. The tissue is next washed in a 0.25 per cent solution of osmic acid, and then in water. After being passed through graded alcohols, the tissue is cleared in xylol, and embedded in paraffin. This method followed by Delafield's hæmatoxylin or methylene blue, counterstained by 0.5 per cent solution of acid fuchsin in 70 per cent solution of alcohol gave excellent results. The chromatin structures of the nucleus and the cytoreticulum appear almost black, while the undifferentiated cytoplasm is red. Acid fuchsin alone without the nuclear stains also yields good preparations.

One objection to Kolossow's solution is that it does not penetrate rapidly enough, and as a result, especially in adult tissue, the structure of the cells in the interior could not be made out as well as that of those in the peripheral portions of sections.

Another killing fluid which gave good results was Gilson's mercuronitric fixing mixture, followed by iron hæmatoxylin for staining. This method produces more uniformly penetrated preparations, but does not show the cytoplasmic reticulum to such good advantage as the osmic acid method.

Hermann's platino-aceto-osmic mixture was also tried. After treatment with this solution the tissue is stained with alcoholic safranin for 24-28 hours. The preparation is then treated with gentian violet according to Gram's method. This method was not as satisfactory as either of the foregoing.

Picrosulphuric-acetic acid was used with good results where general structure was the object sought. This fluid is made by adding a 5 per cent solution of acetic acid to Kleinenberg's picrosulphuric acid solution. Treatment with the above is followed by staining successively with carmalum and Delafield's hæmatoxylin.

Besides section methods, maceration was employed. This process was used with satisfactory results in studying the general structure of the entire cell of the adult tissue. Of the various methods tried, treatment for twenty-four hours with a 20 per cent solution of nitric acid, followed by staining with Delafield's hæmatoxylin and acid fuchsin, gave the best preparations.

The Adult Heart-Muscle Cell.

Examination of the adult tissue shows it to be composed of an anastomosing network of fibers, the demarcation of which into cells is rather uncertain. Apparently a fiber is arranged into cylindrical cells the length of which exceeds many times the diameter. The tapered ends of the cells seem to fit together much in the manner of a dove-tailed joint. The nucleus is oblong in shape and occupies a more or less central position, although it is sometimes seen very close to the periphery of the fiber.

The contractile substance, the fibril bundles, consists of deeply staining, longitudinal masses running the length of the cell. Surrounding the fibril bundles and separating each one is the undifferentiated sarcoplasm. Fig. 15 represents portions of two fibril bundles, each with its envelope of sarcoplasm, from the outer wall of the ventricle of the adult fowl. The fibril bundles are divided by cross striations at right angles to the longitudinal axis into alternating broad, deeply staining bands, and narrower bands more lightly stained (Fig. 15, *bl*). The broad, heavily stained bands correspond to the "Querscheibe," or "Brücke's doubly refractive substance" of mammalian heart muscle (MacCallum, **97**). One of these bands is shown in Fig. 15, *a*. By carefully focussing up and down, a very narrow, deeply staining band can be seen crossing the light bands. This is the "Zwischenscheibe," or "Krause's membrane," which structure is not very distinct nor readily made out. The continuation of this line, however, can be plainly seen in the sarcoplasm surrounding the fibril bundle (Fig. 15, *c*). The Querscheibe, as is readily shown in Fig. 15, are slightly larger in diameter than the more lightly stained parts between.

The sarcoplasm surrounding the fibril bundles is not homogeneous, but is divided into disc-like parts (Fig. 15, *d*), which could be especially well seen in peripheral cells when partially macerated. In preparations of this kind, the discs were found separate from each other, while the fibril bundles remained intact, that is, they did not break up into corresponding lengths. This it seems would militate against the idea of

Krause's membrane being continuous with the line of demarcation between two adjacent sarcoplasmic discs. If the latter were the case, it would seem that the fibril bundles should show a tendency to break and separate along the line of Krause's membrane. This did not happen. Hence it may be that what appears to be Krause's membrane may only be the line of demarcation between two successive sarcoplasmic discs seen through the lightly staining parts of the fibril bundles.

Teased tissue showed the fibril bundles each to be made up of smaller longitudinally disposed parts, the fibrils. The fibril bundles were in all cases surrounded by sarcoplasmic discs.

In the adult tissue the fibril bundles are more abundant in the periphery of the cell than toward the center, which is composed for the most part of a network of undifferentiated protoplasm (Fig. 16). This figure represents a longitudinal section through the center of the cell. Part of the sarcoplasmic discs of a fibril bundle is marked *sa* in this figure.

Cross sections show cells of widely varying diameter. Fig. 17 represents three cells of a medium type. Cells of three and four times the diameter of these are also found. Fig. 17 is exceptional in that it shows the cell boundaries very distinctly. In most cases the cells are so closely applied that it is difficult to distinguish cell walls. These sections show the fibril bundles as dark, deeply staining patches *a*, surrounded by the sarcoplasmic discs. (*sb*) (Fig. 17). Some of these discs are seen to be further subdivided (*sd*), into what MacCallum, **97**, has described as "small sarcoplasmic discs."

Between the cells as represented in Fig. 17, and connecting them, may be noticed a number of threads *t*, which resemble very much the strands of the reticular structure of the interior of the cell. Just what the origin and nature of these structures are, could not be determined. A study of these structures would doubtless throw some light on the question of whether the heart muscle fiber is a syncytium or not. No structure analogous to the "protoplasmic bridges" of mammalian heart tissue or the "stratum granulosum terminale" (Prezwoski, as quoted by MacCallum, **97**, p. 611), of human heart muscle, was found connecting the ends of the cells.

Such, in brief, are the structures met with in the heart muscle cell of the adult chick.

Embryonic Development.

To study the different stages through which the cell passes in its development, sections from embryos varying in length from 8 mm. (15 somites), to 22 mm. (8 days), were examined. Cells characterized by

a certain structure are not confined to any one stage. In the following account cells are said to belong to a certain stage because they were first noticed in that stage, although they may be, and frequently are, seen in sections from tissue several days older.

Thirty hours (15-somite stage).—Cells of this stage exhibit in longitudinal section a characteristic short, broad, cylindrical form (Fig. 1). The ends of the cell taper rather abruptly from the center. The nucleus (Fig. 1, *d*) is large and oval; its short diameter being but slightly less than the diameter of the cell. Large chromatin masses (Fig. 1, *b*) may be seen as heavily stained, irregularly shaped clumps, and a very fine network traverses the nucleus.

The cytoplasm exhibits a pronounced reticular structure, with large and irregular meshes. At the intersections of the threads of this network, the staining is somewhat heavier, marking off these parts more distinctly than the rest of the reticulum.

Fig. 2 is a cross section through the region of the end of the nucleus. The cytoplasm shows the same reticular structure, the nucleus appearing oval in outline.

Cells of this general description are typical of the heart tissue in its very earliest state of formation. They can be readily distinguished from the unmodified mesenchyme cells, in that they do not show the branched structure of the latter.

Seventy-two hours (3-day stage).—Up to this period in its development, the cell differs but slightly from the description given above. At this time, however, several changes are to be noticed. The cell has become larger and the tapering ends have increased in length (Fig. 3). The points of intersection of the threads of the cyto-reticulum are more distinctly marked than before by accumulations of heavily staining material, spherical in form (Fig. 3, *a*). These are also shown in cross sections (Fig. 4, *a*). The nucleus is oval in form though somewhat smaller than in earlier stages. The chromatin masses (Fig. 3, *b*; Fig. 4, *b*) have decreased in size.

Ninety-six hours (4-day stage).—Fig. 5 represents a longitudinal section of a cell of this period. The cell has increased enormously in size, especially in its longer diameter. The deposits on the cyto-reticulum are larger and stand out very clearly and distinctly (Fig. 5, *a*). The meshes of the cyto-reticulum are still very irregular in form. The nucleus does not seem to have undergone the same increase as the cytoplasm, and its chromatin masses have become smaller and more evenly distributed (Fig. 5, *b;* Fig. 7, *b*). Fig. 6 shows a cross section above

or below the nucleus. Some of the meshes of the cytoplasmic network are divided into smaller parts, the small sarcoplasmic discs of MacCallum, 97 (Fig. 6, *sd*).

120-130-hours' stage.—In Figs. 8 and 9 are shown characteristic longitudinal sections of the cell at this stage in its development. The cyto-reticulum has undergone a striking change. Instead of the irregular structure of the preceding stages, we find a definite arrangement of the network into rectangular meshes. The deposits on the reticulum have increased in bulk in such a way that in longitudinal section they appear as oval-shaped bodies (Fig. 9, *a*). In Fig. 9, which represents a slight advance in the development of the cell over that shown in Fig. 8, interposed between every two adjacent longitudinal threads of the network bearing the deposits, is to be seen a longitudinal thread the intersections of which with the transverse threads are not marked by heavily staining deposits (Fig. 9, *bx*).

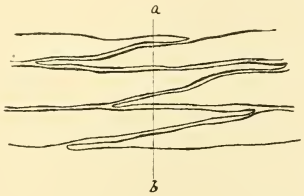
This re-arrangement of the meshwork is apparently the first step in the laying down of the fibril bundles. Subsequent development shows that the longitudinal strands bearing the deposits represent the axes of the fibril bundles, and the deposits, the Querscheibe of the adult tissue. The transverse threads (Fig. 9, *c*) according to MacCallum, 98, give rise (in the mammal) to Krause's membrane. However, this will be referred to again later.

Cross sections (Fig. 10) show the meshes of the reticulum to be further subdivided, but few of the original size remaining. It is to be noted that one or two very large, irregularly shaped meshes are present in this section (Fig. 10, *la*). Apparently these areas, in later stages, become divided into smaller parts, just as the neighboring cytoplasm has already become divided. Evidently this is the manner in which the growth process in the cell takes place, the cytoplasm in the smaller meshes increasing greatly in bulk and then, by subdivision, producing a number of meshes approximately the size of the first.

The nucleus is oblong in longitudinal section (Fig. 8, *d;* Fig. 9, *d*), and roughly circular in cross section (Fig. 10, *d*). The chromatin masses are small, although Fig. 10 shows one of considerable size. It appears that Eycleshymer, 04, in his work on skeletal muscle-cells of Necturus, found just the opposite change to take place in the size and distribution of the chromatin granules, *i. e.,* in younger stages the karyosomes were evenly distributed in the nucleus, and in later stages collected in large masses.

130-140-hours' stage.—The most interesting phase of the entire devel-

opment is seen at this period. The evidence met with in this stage furnishes the most decisive proof in favor of a definite relationship existing between the cyto-reticulum of the embryonic cell and the fibril bundles of the adult. Longitudinal sections present a very marked appearance of cross striations (Fig. 11, *a;* Fig. 12, *a*). On examination it is found that these striations are produced by a growth, principally in length, of the deposits on the cyto-reticulum of previous stages (Fig. 9, *a;* Fig. 3, *a*). These heavily-stained bands stand out as clearly as if stamped with a die. Sections at this period present all gradations of striations as may be seen from Figs. 11 and 12. The transverse strands of the cyto-reticulum are not very prominent. The tapering ends of the cells show an enormous increase in length. It is to be noted that the striations first appear in the elongated ends, at least they show a greater degree of development in this part of the cell. These markings



cause the ends of the cell to stand out more prominently than the other regions, and it is now very difficult to distingush cell walls in longitudinal sections. However, by means of the heavy striations, one can see how these slender projecting ends have made their way between other cells, and thus trace the formation of the syncytium-like structure of the adult tissue. In later stages, when the cell exhibits uniform striation, the course of the ends of the cells cannot be so well followed. The accompanying diagram would then represent the structure of the adult fiber in longitudinal section. This would also explain the fact that a cross section of the adult tissue, as for example, from *a* to *b*, shows cells of widely varying diameter. A similar explanation was offered by MacCallum, **98**, in the case of the striated muscle of the pig.

Fig. 12 shows the ends of two adjacent cells seen in the same section as Fig. 11, but which have apparently proceeded further in their development. The striations are well marked. In the figure several fibril bundles are represented (*a*). The structure found here corroborates the statement that the formation of the Querscheibe starts in the ends of the cells.

Cross sections (Figs. 13 and 14) show an increased number of the smaller meshes of the cyto-reticulum, while the deeply staining deposits (*a*) which are cross sections of fibril bundles have become greatly enlarged. These heavily staining patches are best developed toward the periphery of the cell. The nucleus is roughly oval in outline.

From this structure to that of the adult is but a step. While all the intermediate stages between this and the adult were not examined, sections from stages beyond this up to the eighth day, showed the same structure in various degrees of development. In the adult the sarcoplasmic discs are better developed. The Querscheibe are broader and resemble thick, flat plates. The length and diameter of the cell is greatly increased.

SUMMARY.

The facts which appear to be of importance as set forth in the foregoing, are as follows:

1. The cytoplasm of the early embryonic heart cell is traversed by an irregular network, the nodes of which are marked by heavily staining deposits.

2. This network tends to become more and more regular, until its strands are longitudinally and transversely disposed.

3. The heavily staining deposits on the primitive network develop into the Querscheibe of the adult fibril bundle.

4. The longitudinally disposed lines of the network represent the axes of the fibril bundles of the adult.

5. The sarcoplasmic discs of the adult develop from the inter-reticular cytoplasm of the embryonic cell.

General Discussion on the Relation Between the Cytoplasm and the Fibril Bundles.

According to Ranvier, 89, the myocardium of the mammal is composed of rhomboidal branching cells. The nucleus is centrally placed and is surrounded by a granular mass stretching out in the axis of the cell. Surrounding this granular mass is the contractile element which shows longitudinal and transverse markings. This element is the fibril and is made up of successive segments having the same structure as voluntary muscle.

Koelliker, 02, describes the mammalian heart muscle as composed of an interlacing network of cells having centrally placed nuclei. The contractile substance, as in voluntary muscle, consists of fibril bundles, the so-called "Muskelsäulchen," which show a definite transverse striping.

Of the later workers, Godlewski's, 02, description is interesting, because of his denial of cell structures in the heart muscle of the rabbit. According to him the heart muscle is a syncytium in which there is no cell demarcation. The contractile substance is the fibril which shows the various markings described by other authors.

Thus, in general, in addition to the above, the work of investigators goes to show that the contractile substance in the muscle tissue is the fibril. A number of these in turn compose the fibril bundle. Workers are not agreed as to the origin of the fibril bundles. The older hypothesis that they are extra-cellular structures is now refuted and the generally accepted opinion is that they are intracellular.

Two current views exist as to the nature of the fibril bundles. The first is that these structures are coagulation products, and that the living cell contains neither cyto-reticulum nor fibrils (Englemann, 73-81). The second view is that the fibrils are differentiated structures which are formed in the living cell. The latter theory is the one which the results of many workers seem to verify. Of the latest workers Eycleshymer, 04, reports having observed the fibrillæ (fibrils) in the living muscle cells of larval necturus.

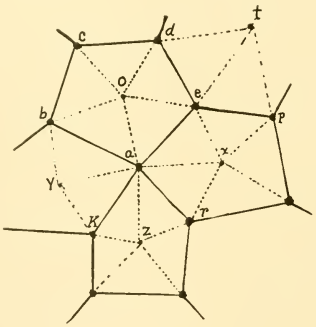
Concerning the origin of the fibrils, there are what is known as the network theory and the fibrillar theory. The upholders of the network theory maintain that the muscle cell contains a contractile reticulum, the longitudinal threads of which form the fibrils, the meshes being filled with a more fluid substance. Others consider that the fibrils are produced by the coagulation of the fluid substance, as a result of the action of various reagents. Later work apparently refutes the latter idea. The advocates of the fibrillar theory maintain that the fibrils are the contractile elements, and further, that they arise independently of the cyto-reticulum. Eycleshymer's, 04, work on necturus, supports this idea, and Godlewski, 02, in his work on the striated muscle cell of the rabbit, has been able to find no trace of a cytoplasmic network.

In the theory urged by MacCallum, 98, we have a combination of the network and the fibrillar theories. In this author's words (p. 211), "It simplifies the conception of the structure of striated muscle fiber greatly, to consider the fibril bundles and the membranes bounding the compartments in the sarcoplasm as derived from the primitive network found in the muscle cells of very young embryos." (p. 209) "This network tends to become more and more regular until the meshes are of the form of large discs. Some of these break up into smaller ones and in the nodal points of the network there is an accumulation or differentiation

of its substance, giving rise to longitudinally disposed masses. These become what in the adult are known as fibril bundles and the discs are the sarcoplasmic discs."

Now the question is, How do these theories apply to the conditions met with in the heart muscle of the chick? We will consider MacCallum's theory first.

The occurrence of the breaking up of the meshes of the cyto-reticulum into smaller parts was noticed in the case of the chick. That the fibril bundles arise from the center of the meshes at the nodal points of the network appears, however, to be untrue. Here, in fact, it seems that the nodes of the original network mark the positions of the fibril bundles; in other words, that the primitive longitudinal threads develop into these structures. To illustrate, the accompanying figure represents a diagrammatic cross-section of the cyto-reticulum. The unbroken lines represent the threads of the original network. The circular masses at the inter-



sections of this network (*a, b, c,* etc.) represent the heavily-stained deposits. The dotted lines divide the large meshes or discs into the "small sarcoplasmic discs" of MacCallum (*boc. cod.* etc.). Now according to the author just mentioned, a fibril bundle arises in the center of any large disc at the point of intersection of the dotted lines (*o, x,* etc.). The full lines would then represent boundaries of sarcoplasmic discs. However, in the case of the chick the facts seem to indicate that the fibril bundles arise at the points of intersection of the lines of the original network (*a, e, b,* etc.). Then the sarcoplasmic disc surrounding any one particular fibril bundle, as, for example, that one the cross section of which is represented by *a,* would be the area bounded by *yboexrzk.*

The writer's preparations clearly show that the nodal points of the original cyto-reticulum of the embryonic heart cell are marked by more heavily staining deposits, both in longitudinal and in cross section (Figs. 3, 4, 5, 6). The deposits can be followed in successive stages, and are always identified with the longitudinal and transverse threads of the network. Eventually they develop into the Querscheibe, and the longitudinal threads of the network become the axes of the fibril bundles of the adult tissue. If this be true, the fibril bundles cannot originate from the centers of the meshes seen in cross sections of the cyto-reticulum.

Suppose now that fibril bundles were to form at the points *a* and *e* of the diagram. Then we would have the two fibril bundles surrounded by one set of sarcoplasmic discs (*odtpxrzkyb*), a condition sometimes found in the adult; also mentioned by MacCallum, **97** (p. 613), in the human heart muscle. However, were these two sets of sarcoplasmic discs to become separated by a plane of division along a line from *o* to *x*, then each fibril bundle would have its own set of sarcoplasmic discs, the structure more generally met with in the adult.

If the fibril bundle in its development follows the method suggested by the writer, another difficulty is encountered with the results of MacCallum's, **98**, work. This worker states that the transverse membranes of the cytoplasmic reticulum of the myoblasts of man and of pig (represented in the chick in Fig. 8, *a;* Fig. 9, *c*), give rise to Krause's membrane in the adult. Attention is called to the fact that (as seen in Figs. 8 and 9) the intersections of these transverse lines with the longitudinal lines of the reticulum mark the positions of the deeply-staining substance which later becomes the Querscheibe. Now, in the adult, Krause's membrane is found as a narrow transverse band across the lightly-staining portions of the fibril bundles, and not at all connected with the Querscheibe. Thus it is readily seen that MacCallum's explanation of the formation of this structure does not apply in the case of the chick, nor could its origin be determined satisfactorily, for, as was remarked in another place, it is not very well differentiated in the heart muscle of the adult chick.

At this point it is interesting to consider the work of Eycleshymer, **04**, on the striated muscle cell of Necturus. This author states that in the study of the striated muscle cells of Necturus, he has been unable to find any evidence of a definite or fixed relation between the cytoplasmic network and the fibrillæ (fibrils). Further, as serious objection to the existence of such a relation, he says that the fibrillæ are unstriated for some time after their appearance.

In the case of the chick, however, the above is not true. For if we consider the longitudinal threads of the network of the stage represented in Figs. 8 and 9, as the incipient fibril bundles, then the deposits marking the intersections of the threads would represent the striations, since later these develop into the Querscheibe of the adult tissue. The question is just what is to be understood by "first appearance of fibrillæ." If by this we mean the earliest stage in the development of the fibril bundles at which there is any resemblance to the adult structure, we may say that the fibrillæ are striated from the start in the chick.

With reference to another point in this connection, the writer here quotes a passage from the same author, pp. 298, 299: "A point of capital importance is found in the fact that in Necturus, Amia Lepidosteus . . . . as my own observations show, and in other forms as Kaestner, 92, has found, the beginning of fibrillation is coincident with the first contractions. The movements of the embryo first begin in the anterior of the mid-dorsal myotomes and in these the myoblasts are first fibrillated. The above considerations led the writer to support the theory that the fibrillæ are pre-existent structures and represent the principal contractile element."

The same argument cannot be applied to the heart muscle of the chick, because the first contractions occur at the time when about 15-17 myotomes have been formed in the embryo. As may be seen from the representation of the heart muscle cell at this stage (Fig. 1), no structure which might be truly called a fibril is present. The first appearance of anything that in the faintest way resembles the adult is not seen before the 120-130-hours' stage (Figs. 8 and 9).

If the fibrillæ are not pre-existent structures, what then are the contractile elements in the very early embryonic stages? In view of the above facts the explanation offered by MacCallum, 97 (p. 620), seems plausible. This author suggests that the contractile elements in the early embryonic heart would be represented by the irregular network seen at that stage before true fibrils exist. This, it seems, would lend support to the writer's suggestion in regard to the origin of the fibrils. For if, as MacCallum says, the cytoplasmic network represents the contractile element in the early stages, it seems reasonable at least to consider the longitudinal lines of this network as developing into the fibrils rather than to suppose the latter to arise from accumulations of the network-substance in the cytoplasm contained between the meshes.

In conclusion, the results of the work embodied in this paper point to the existence of a definite relationship between the cytoplasmic reticulum of the early embryonic cell and the fibril bundles of the adult cell.

LITERATURE CITED.

Engelmann, Th. W., 73-81.—Mikroskopische Untersuchungen über die quergestreiften Muskelsubstanz. I u. II Pflügers Arch., Bd. VII, 1873.

——— Kontraktilität und Doppelbrechung. Pflügers Arch., Bd. XI.

——— Neue Untersuchungen über die mikroskopischen Vorgänge bei der Muskelkontraktion. Pflügers Arch., Bd. XVIII, 1878.

——— Mikrometrische Untersuchungen an kontrahierten Muskelfasern. Pflügers Arch., Bd. 26, 1881.

——— Bemerkungen zu einen Aufsatz von Fr. Merkel: Ueber die kontraktion der quergestreiften Muskelfaser. Pflügers Arch., Bd. 26, 1881.

——— Ueber den faserigen Bau der kontraktilen Substanz mit besonderer Berücksichtigung der glatten und doppelt schrag gestreiften Muskelfasern. Pflügers Arch., Bd. 25, 1881.

Eycleshymer, A. C., 04.—The Cytoplasmic and Nuclear Changes in the Striated Muscle Cell of Necturus. Am. Journ. Anat., Vol. III, No. 3.

Godlewski, E., 02.—Die Entwickelung des Skelet- und Hertzmuskelgewebe der Säugethiere. Arch. f. mikr. Anat., Bonn, 1902, Bd. LX.

Koelliker, 02.—A. Koelliker's Handbuch der Gewebelehre des Menchen. 6. Anlage, p. 609, Leipz., 1902.

Kolossow, A., 92.—Ueber eine neue Methode der Bearbeitung der Gewebe mit Osmiumsäure. Zeitschr. f. wissenschaftl. Mikroskopie Bd. 9, 1892, p. 38.

MacCallum, J. B., 97.—On the Histology and Histogenesis of the Heart Muscle Cell. Anat. Anz., Jena, 1897, Bd. XIII, S. 609-620.

——— 98.—On the Histogenesis of the Striated Muscle Fiber and the Growth of the Human Sartorius Muscle. Johns Hopkins Hosp. Bull., Balto., 1898, Vol. IX, pp. 208-215.

Ranvier, 89.—Traité technique d'histologie. Paris, 1889.

EXPLANATION OF FIGURES 1 TO 17.

Abbreviations Used.

a = Heavily staining deposits at the nodal points of cyto-reticulum of early embryonic cells.

b = Karyosome masses.

c = Transverse threads of cyto-reticulum.

d = Nucleus.

bl = Lightly staining bands on the fibril bundles.

sd = Small sarcoplasmic discs of MacCallum, 97.

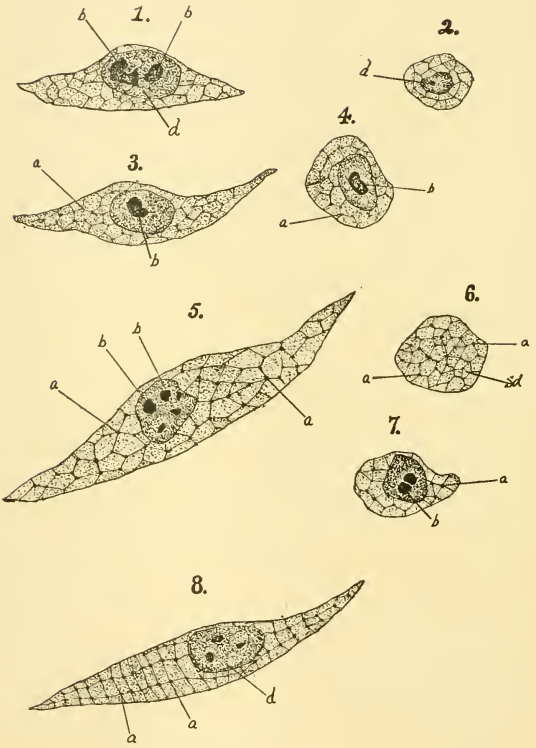
sb = Sarcoplasmic disc.

sa = Part of a sarcoplasmic disc seen in longitudinal section.

bn = Cyto-reticulum of the interior of the adult cell.

bx = Points of intersection of longitudinal threads with transverse threads, not marked by heavily staining deposits.

la = Enlarged cytoplasmic area.



FIGS. 1 TO 8.

The accompanying figures are camera lucida drawings made at table level. In all cases the magnification is about 2000 diameters.

FIG. 1. Longitudinal section of heart muscle cell from a 30-hour embryo. The cytoplasm shows an irregular network.

FIG. 2. Cross section at 30-hour stage.

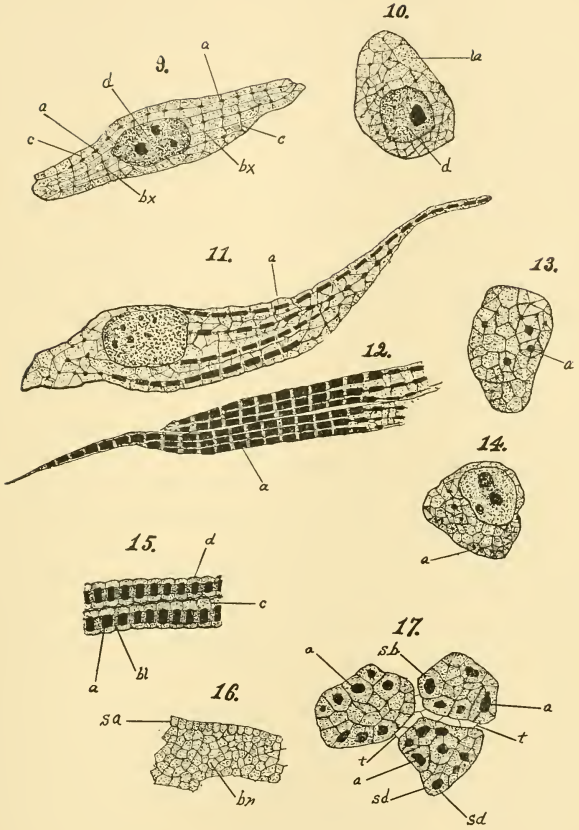
FIG. 3. Longitudinal section, 72-hour stage.

FIG. 4. Cross section, 72-hour stage.

FIG. 5. Longitudinal section, 96-hour stage.

FIGS 6 and 7. Cross sections, 96-hour stage; *Fig. 6*, above or below the nucleus; *Fig.* 7, through the region of the nucleus.

FIG. 8. Longitudinal section, 120-130-hour stage.



FIGS. 9 TO 17.

The accompanying figures are camera lucida drawings made at table level. In all cases the magnification is about 2000 diameters.

FIG. 9. Longitudinal section, 120-130-hour stage.

FIG. 10. Cross section, 120-130-hour stage.

FIGS. 11 and 12. Longitudinal sections, 130-140-hour stage.

FIGS. 13 and 14. Cross sections, 130-140-hour stage.

FIG. 15. Longitudinal section of two adjacent fibril bundles of adult tissue.

FIG. 16. Longitudinal section through the reticular structure found in the center of the adult cell.

FIG. 17. Cross section of the adult heart muscle cell.

A STUDY OF THE STRUCTURE OF THE GASTRIC GLANDS OF THE DOG AND OF THE CHANGES WHICH THEY UNDERGO AFTER GASTROENTEROSTOMY AND OCCLUSION OF THE PYLORUS.

BY

BASIL C. H. HARVEY,

Hull Laboratory of Anatomy, University of Chicago.

WITH 5 FIGURES.

New methods of fixation and staining introduced by Bensley in his work on the alimentary tract, have demonstrated new facts in the minute anatomy of this region. They have been especially valuable in their application to the study of the cellular elements of the various digestive glands, and he has already applied them to the study of the stomach in many animals. His work was confined, however, to normal anatomy, and by this investigation of the structure of the mucous membrane when placed under abnormal conditions, and subjected to the action of external influences different from those normally acting upon it, it was hoped, with the assistance of these improved methods, to learn something which might still further advance our knowledge of the nature and relations of its highly differentiated cells.

TECHNIQUE.

The methods of investigation adopted were as follows. The dogs were killed by illuminating gas at various times after operation, always when they were in the fasting condition, at least ten hours after feeding. The stomach was immediately opened and its condition noted. Strips of mucosa at the sites selected for study were then removed. Parallel incisions 1 cm. apart were made with a razor through the tunica mucosa, so as to isolate strips 1 cm. wide and 2 cm. long, including 1 cm. of gastric and 1 cm. of duodenal mucous membrane. These were then dissected free from the muscular coat with razor and scissors, and laid down in a drop of fixing fluid on a piece of sheet cork with the free surface next the cork. It was pinned in place by porcupine quills, and

a little fixing fluid applied to the muscularis mucosæ by a pipette. After two or three minutes the pins were removed and the strip dropped into a bottle of the fixative. This method prevents the curling of the strip due to the contraction of the muscularis mucosæ, which otherwise disturbs the relations of the glands.

Small pieces of mucous membrane were removed at the time of the operations and fixed in various fluids. These served as controls with which glands modified as the result of the operations were compared later, and also as material in which the normal, healthy structure of the mucous membrane was studied.

Experiments were made with very many fixing fluids and it was found that the one which gave the best all-around results was made of formalin, 3 per cent aqueous solution of potassium bichromate, saturated aqueous solution of mercuric chloride, and water, in equal parts. Strips of mucosa were left for two hours in this fluid, then washed and dehydrated. It is difficult to fix both the zymogen granules and the cytoplasm in the cells of a dog's gastric glands. The zymogen granules are more difficult to fix than in cats or rabbits, and are not fixed at all in Zenker's fluid, and very poorly in Bensley's fluid or Bouin's. Kopsch's fluid fixes the granules well and the cytoplasm poorly. Bensley's and Bouin's fluids fix the cytoplasm well but the zymogen granules poorly. The above solution gave very good results for both.

Paraffin sections three or four micra thick were cut, and fixed to the slide by the water method. The following stains were found most instructive. Neutral gentian as used by Bensley, 00, gives a very definite blue stain of the zymogen granules. In material fixed in Bensley's fluid it does not stain the prozymogen, but in formalin fixations I have usually found it to stain the prozymogen purple. A mixture of equal parts of saturated aqueous solutions of acid fuchsin and orange *G* stains the granules of the parietal cells a definite pink in a minute or two, and if it be followed by a saturated aqueous solution of toluidine blue for one or two minutes, there is produced in addition a very pretty metachromatic pink tint in the surface mucus and in the thecæ of the cells of the surface and stomach pits, and also a deep blue color of the zymogen granules and the prozymogen. Mayer's muchæmatein and mucicarmine were also used to stain mucus. The former gave the more constant and the latter the more beautiful results. The modifications of its preparation suggested by Rawitz, 99, who evaporated the mixture of carmine, aluminum chloride and water to dryness at a low temperature before dissolving in alcohol, and by Bensley, who used Mayer's stock

solutions instead of the diluted forms which Mayer employed, were used with advantage. The following copper-chrome-hæmatoxylin stain personally communicated to me by Professor Bensley and reported here for the first time, has been very convenient and valuable. Sections are placed for one minute each in a saturated solution of neutral copper acetate, a 3 per cent aqueous solution of potassium bichromate, and a saturated aqueous solution of hæmatoxylin crystals, being washed in tap water after each. This round is once repeated and then the stain is differentiated in Weigert's borax-ferricyanide. It stains the granules of the parietal cells black and this stain is very persistent, not being quickly removed by the borax-ferricyanide. It stains the chromatin black. From the latter, however, the stain quickly disappears in the differentiating solution, leaving the parietal cell granules clearly differentiated (Fig. 5). This method was very satisfactory for the detection and study of parietal cells. Paracarmine and iron-hæmatoxylin were also used as nuclear dyes, and very satisfactory preparations were obtained by following them with muchæmatein and mucicarmine respectively.

Normal Anatomy.

As has been shown by various investigators from Cobelli, 66, on, the entire stomach exclusive of the pars œsophagea may be divided throughout the mammalia into three parts, cardiac, fundus, and pyloric, in accordance with differences in character of the tunica mucosa.

I. *The cardiac region* is occupied by glands which are fully described by Edelmann, 89, Bensley, 02, Haane, 05, and others, and which are not especially concerned in this investigation.

II. *The fundus region* is occupied by mucous membrane which is chocolate pink in color and is composed of glands opening into a cylindrical pit, which is merely a depression of the surface extending through one-fifth to one-fourth of the thickness of the mucous membrane. The epithelium lining it passes by a very gradual transition into that of the general gastric surface on the one side and into that of the gland necks on the other. Its cells are cylindrical, containing a round or oval nucleus in the attached half, and at the free end a theca containing a rounded mass of mucous secretion which becomes thrown out during digestion. Bensley, 98, has described a second globule of mucus near the nucleus in the cat, and Holmgren, 02, and others, describe as trophospongium, structures appearing in these cells between the nucleus and the free end. The cytoplasm in fixed specimens presents a fibrillar element and an interfibrillar substance.

The glands proper are made up of two parts, the necks opening into the foveolæ and the bodies opening into the necks. The neck occupies about two-thirds of the length of glands near the lesser curvature and one-third of those near the greater curvature. There are very considerable individual variations in the relative lengths of these two portions, the variations being due mainly to differences in length of the body. Both parts are made up of parietal cells and chief cells.

(a) *Parietal cells.* These are relatively more numerous at the foveolar end of the neck, and they become relatively less numerous toward the muscularis mucosæ. They have ordinarily a diameter two or three times as great as that of neighboring chief cells. They are separated from the lumen by a layer of chief cells, between which lie the intercellular ducts, putting them in communication with it, although they occasionally extend to the lumen themselves, especially in the neck region. Their outline in sections is usually round or oval. The nucleus is central and is often double or even triple, and there is evidence of direct division of these nuclei without corresponding division of the cytoplasm. (Cade, 01.) This author observed near the nuclei, bodies which he regards as debris of broken-down nuclei which have been replaced by new ones arising by direct nuclear division. The cytoplasm contains many fine granules, staining pink in the fuchsin-orange G mixture, dark brown or black in copper-chrome-hæmatoxylin (Fig. 5), and remaining unstained by neutral gentian or toluidine blue. Some cells are packed with them. Vacuoles, which Sachs, 87, regarded as indications of pathological processes, and which Schmaus and Albrecht, 95, regard as indicative of degeneration, appear in many of my preparations of the apparently healthy resting stomach. A granule free zone, more or less closely surrounding the nucleus and described by Zimmerman, 98, Kolossow, 98, and others, is occasionally present. It, as well as the vacuoles, may be in part due to the presence of the system of intracellular canals demonstrated by Eric Müller, 92, Golgi, 93, and Langendorf and Laserstein, 94, communicating with intercellular ducts or directly with the gland lumen, and appearing in various parts of the cell section as granule free areas.

Spirilla staining purple with neutral gentian, and black with copper-chrome-hæmatoxylin, described first by Bizzorzero, 93, appear constantly in the gland lumina and are closely associated with the parietal cells. According to Theohari and Babes, 05, they were not to be found actually within these cells in dogs till after treatment with their gastrotoxic serum. Bizzozero found them, however, in the healthy stomach and

I have been able to confirm his observations, seeing them frequently in parietal cells, occupying the intracellular canal system, or contained in vacuoles, or sometimes, also, apparently in the cytoplasm itself, although in the latter case it must be borne in mind that they may be in extremely small ramifications of the intracellular canals. They do not appear to have any association whatever with the chief cells.

A few parietal cells appear in all my preparations of material fixed in aqueous bichromate solutions, which take a yellowish brown or yellowish green color with this fixative and take no further stain whatever with acid fuchsin, orange G, Ehrlich's triacid mixture, eosin, mucicarmine, muchæmatein, neutral gentian, or paracarmine. With copper-chrome-hæmatoxylin they stain like other parietal cells but give up the stain very readily, so that in sections properly differentiated they are unstained with this method also. They are unaffected by hydrochloric acid or ferricyanide of potassium even after prolonged exposure, and they do not give the iron reaction of MacCallum. They do not blacken in osmic acid nor take any stain with Sudan III. In material fixed in solutions which do not contain salts of chromic acid they do not appear, the parietal cells being nearly uniform in color and staining reactions. Occasionally they present the full and rounded outline common to parietal cells, but many are irregular in shape and many are shrunken. Their nuclei are spherical and contain a normal amount of chromatin staining in hæmatoxylin. The karyoplasm is yellowish green. The cytoplasm contains fine granules which stain in hæmatoxylin but give up the stain much more readily than the granules of other parietal cells. They retain it, however, more firmly than the cytoplasm about them, so that in sections stained with this method there sometimes appear cells in which the cytoplasm is yellowish green while the granules contained are black. Cells somewhat similar to these were described by Popoff, 98, in material taken from stomachs in inflammatory conditions and fixed in Flemming's fluid. He reports similar staining reactions, but found in the cells karyokinetic figures and net formations which I was quite unable to find in my preparations, although I employed the methods which he recommends. These peculiar parietal cells resemble very closely the chromaffine cells described by Kohn, 01, in the suprarenal gland, the glomus caroticum and in various parts of the sympathetic nervous system. Their staining reactions are similar, the most important one, namely, the selective affinity for salts of chromic acid, being very distinct in my preparations, so that the parietal cells are separated into two classes by it. The granules of these cells in the stomach seem to possess

somewhat less susceptibility of staining with basic dyes than those he describes, and they are not, as in his cells, the only part of the cell structure possessed of the affinity for chrome salts, as is clearly shown in those of my preparations in which the granules are slightly stained by hæmatoxylin while the rest of the cytoplasm is yellowish green. In the last particular they agree with Rabl's, 91, description of the chromaffine cells of the suprarenal gland.

Mitotic divisions are extremely rare among the parietal cells. Tortora, 99, reports their presence in the neck region. Popoff, 97, describes them as occurring in inflammatory conditions. I was not able to find them in any of my preparations.

The parietal cells are sometimes invaded by lymphocytes, which may then appear in the interior of them, as has been reported by Sewall, 79, Hamburger, 89, Bonnet, 93, and others. I have frequently seen them invaded by mast cells, both in healthy glands and in those modified as a result of operations. Stinzing, 99, reports similar observations of mast cells, which were especially abundant twelve hours after ingestion of food.

(b). *Neck Chief Cells.* These have been well described by Bensley, 98, as forming a layer of pyramidal cells immediately surrounding the lumen with their large end lying next it. The nuclei lying near the attached end are oval in cells which have thrown out their secretion, and flattened in those yet full of it. The cytoplasm consists of a trabecular framework containing in its interstices the cell secretion. This is poured out during several hours after ingestion of food, but accumulates during rest near the free end of the cell, where it forms definite spherules in preparations of material fixed in alcoholic solutions, but is precipitated as an irregular mass upon the trabecular framework in material fixed in watery solutions. In whatever form it is present, he has shown that it stains differentially with mucin dyes, pink with mucicarmine, blue with muchæmatein, blue in Ehrlich's indulin-aurantia-eosin blood-staining fluid, and takes a metachromatic pink tint in aqueous solutions of toluidine blue. For these reasons he regards it as mucus. It differs, however, in its reaction to mucus stains, from the mucus in the foveolar cells and on the surface. While occasionally mucus in both regions is stained in the same section, usually when one region is well-stained, the mucus in the other is faintly or not at all stained. Bensley explains this difference on the basis of the multiplicity of the chemical constitution of mucins, which is described by Mayer, 97, and others. The neck chief cells pass by a gradual transition into those of the foveolar

epithelium, the intermediate zone presenting frequently cells undergoing mitotic division, and it is probable, as Bizzozero pointed out, that the cells of both these regions arise in this zone, some new cells forming foveolar epithelium, and some passing into the gland to become neck chief cells.

(c). *Body chief cells.* These are similar in shape to the neck chief cells though somewhat larger. The spherical nucleus near the attached end is never flattened by accumulated secretion. The cytoplasm contains a fibrillar reticulum presenting a strong affinity for basic stains due, as Bensley has shown, to the presence in it of a substance particularly concentrated in the attached end of the cell, giving a strong reaction for masked iron and corresponding in this respect to the prozymogen demonstrated by MacCallum in the pancreatic and other glandular cells. It often presents the appearance of bands extending toward the nucleus, staining dark brown or black with copper-chrome-hæmatoxylin and intensely blue with toluidine blue (basal filaments of Solger, 96, ergastoplasm of Garnier, 97, and Cade, 01). At the free ends of these cells are many coarse granules occupying meshes formed by the cytoplasmic trabeculæ. They stain blue with toluidine blue, as does also the cytoplasm, but they are stained differentially an intense blue by neutral gentian. These are the zymogen granules of Langley, 81.

In my preparations I was quite unable to find mitotic divisions of the body chief cells, a result agreeing with that reported by Cade, 01. Bizzozero, 93, reports that in young dogs he found them occasionally, but extraordinarily seldom.

There is no gradual transition from neck chief cells to body chief cells but an abrupt change, and cells possessing fully the characters of ferment cells lie next to cells possessing fully the characters of mucous cells. The two varieties were regarded by Bensley, 96, and Zimmermann, 98, as specifically distinct. No part of the body chief cells is stained by mucus-staining dyes, and therefore it may be concluded that they take no part in the formation of mucus. They are specialized for the formation of ferment. In this function there is no evidence to show that the neck chief cells have any part unless the presence of a very faint iron reaction in their bases may be interpreted as such. They are specialized for the formation of mucus. Trinkler, 83, reports that he saw what seemed to be transition stages, and Cade, 01, states that in the zone where the body of the gland meets the neck he observed what he thought might be transition forms, but says they were not sufficiently distinct and definite to permit of a positive statement of such transition.

Bensley and Zimmermann did not find them, and my own observations have been quite in accord with theirs. No chief cells appear in any of my preparations made by the methods introduced by Bensley, which are not either clearly mucous or clearly ferment cells.

While the body chief cells are almost exclusively ferment cells, there exist among them occasional though rare mucous cells. Somewhat similar cells described by Trinkler, 83, were interpreted by him as transition forms between parietal and chief cells, but as Bensley has pointed out, the selective action of mucicarmine demonstrates clearly their true nature.

III. *The pyloric region* is occupied by mucous membrane, which is whitish yellow in color. It extended in several dogs to a distance of 6 to 8 cm. from the pyloric valve, the extent being usually about 1 cm. greater along the major curvature than along the minor. These measurements agree with those reported by Deimler, 04, for the same animal. When long strips of mucous membrane extending from the pyloric valve into the fundus region are removed from the stomachs of dogs and flattened out, the distance from the pyloric valve to undoubted fundus mucosa is from 10 to 12 cm. An intermediate zone 2 to 3 cm. wide exists between the two regions. In the pyloric region the foveolæ are wider than in the fundus region and each extends through from one-third to two-thirds of the thickness of the mucous membrane and each, as has been shown by the reconstructions of DeWitt, receives the openings of several branched glands. These have a larger lumen than the fundus glands, and are lined by cells of one kind only which resemble accurately the neck chief cells. Like them they secrete mucus accumulating during rest in spherules in the free end of the cell, flattening the nucleus against the attached end and being poured out after ingestion of food. It occupies the meshes of a delicate cytoplasmic framework. There appear among these cells, as Hamburger, 89, has pointed out, the small pointed cells of Stöhr which Bensley found also among the neck chief cells of the fundus region. Stöhr, 82, describes also the occasional presence of parietal cells in the pyloric glands of man. I did not find them in my preparations of the dog's stomach.

In the intermediate zone, as one studies parts successively nearer the pylorus, the parietal and ferment cells gradually become fewer and disappear. The disappearance of the two kinds of cells seems to proceed *pari passu,* and I did not find the parietal cells extending more than one or two glands beyond those in which ferment cells are to be found. Across this zone the gland bodies become progressively shorter

till they disappear, while the length of the gland necks is very little altered. From a study of the intermediate zone it appears that the pyloric glands correspond to the neck region of the fundus gland without the parietal cells.

Cade's Experiments and Observations.

In 1901, in a paper dealing with the normal anatomy of the gastric mucous membrane, and with the changes which it undergoes in various physiological conditions and after its subjection to various operative procedures, Cade gives an account of the alterations in its structure in a dog and a cat near the site of gastroenterosomies which he had performed. His observations were made on only one animal of each species and the changes were studied between six and seven months after the operation. In the dog he effected a gastrojejunostomy connecting with the stomach in the fundus region, by which part of the food might pass into the intestine, but since the normal pylorus remained open, part of the food might pass by the normal way. In the cat he made a similar anastomosis, and in addition divided the mucous membrane in the middle of the stomach, making two sacs, one proximal communicating with the œsophagus and jejunum, and the other distal communicating by way of the pylorus with the duodenum but never containing any food. The animals were killed after six and a half months and the changes at the site of the gastroenterostomies studied.

He found the foveolæ at the wound margin enlarged and deepened, due to the absorption into them of parts of the gland necks. In their deeper parts were seen occasionally cyst-like dilatations, the walls of which were made up of cells shorter and flatter than those normally constituting this epithelium. Otherwise they were unchanged.

The glands were sinuous in outline and their lumina irregularly enlarged. The gland cells were of one kind only and resembled closely the neck chief cells of normal glands. They were cylindrical or cubical in outline, with nuclei poor in chromatin and often flattened against the attached ends of the cells. The cytoplasm was formed of a network, with fine meshes and stained a light pink tint with carbol-thionin, similar to, but fainter than that which he observed with this dye in pyloric gland cells and other undoubtedly mucous cells. They did not, however, take the metachromatic pink tint with toluidine blue which he obtained in clearly mucous cells. They did not take any stain at all in muchæmatein, mucicarmine, or indulin, but did not differ in this respect from normal neck chief cells or pyloric gland cells, for Cade did not

obtain satisfactory results with mucous staining dyes in any of these cells. Toluidine blue failed completely to demonstrate the presence of prozymogen or zymogen granules.

Notwithstanding the failure of these cells to take any stain with mucous staining dyes, he considers the glands so modified to be muciparous in function and similar to normal pyloric glands, basing his conclusion upon their general appearance with large lumina and sinuous outlines, upon their connection with large foveolæ, upon similarities of structure of their cells, and upon the absence from them of zymogen or prozymogen. He finds such modifications only in the immediate vicinity of the anastomosis. At some distance from it the mucous membrane is unaltered and preserves all the characters of normal fundus mucosa. Between this normal mucosa and the line of operation is a transition zone in which as the area examined is progressively farther from the new pylorus, the following changes were observed: The lumina become successively narrower and more regular; parietal cells appear, at first pale and poor in granules, gradually becoming more granular and richer in nuclear chromatin. Ferment cells appear in the ends of the glands with zymogen staining in toluidine blue and prozymogen staining in Bismarck brown, constituting a steadily increasing proportion of the chief cells of the gland body until the structure normally present in the fundus region is attained.

His observations may be summarized in the statement that at the site of a gastroenterostomy made in the fundus region, there is formed a new pylorus which is similar to the normal pylorus in the general form of the glands present, in their relation to the foveolæ and in the character of the glandular elements. Upon this observation he bases the conclusion that these structures possess a morphological flexibility and an ability to transform themselves in response to the influence of new and altered conditions of existence and to assume the performance of new functions quite different from those which they usually perform.

Discussion of Cade's Work.

These observations and conclusions have bearings upon several important theories, first, that of the specificity of cells. They indicate that for these cells, at any rate, specificity in the sense in which Bard, 98, uses the term, does not exist. The second is that of the cause of differentiation of the pyloric mucous membrane. If it can be produced at will by the surgeon from fundus mucosa by subjecting it to the influence of mechanical conditions which he can at any time induce, a strong

suggestion is afforded that somewhat similar mechanical influences, naturally produced, may account for the ontogenetic production of this special form of mucosa. Quite in accord with this suggestion is that offered by Bensley, **02**, as explanation of the differentiation of the cardiac mucous membrane. He thinks it may be effected by the action of mechanical forces naturally produced, operating through many successive generations and associated with natural selection.

Cade's work, while extremely important and significant, seemed to leave unanswered several questions which naturally arise in connection with it. First: Are these changes which he observed constant and permanent? He observed them in a single dog and a single cat, and at only one stage after operation, namely, that of six and a half months. He believed they were permanent, but lamented the lack of material in which to study later stages. He was quite sure, however, that at such stages the changes would be yet more marked than in those which he studied. Second: What becomes of the parietal cells which have disappeared? Do they become disintegrated or transformed into other cells? No suggestion of an answer to these questions was offered. Third: What becomes of the ferment cells which disappear? Are they disintegrated or transformed? He thought they were transformed into the mucous cells which replaced them, but no evidence beyond the fact of replacement is adduced. Fourth: Whence come the new mucous cells which occupy their places? He believed they arose by transformation of the ferment cells, but an equally legitimate conclusion, as far as his experiment could explain the question, would be that they arose by the division of previously existing mucous cells found constantly, though in small numbers, in the gland bodies. It is true that evidences of division in these cells are reported as being exceedingly rare by Bizzozero, **93**, and that none were actually found in Cade's preparations, but it seems quite possible that the stimulus of the operation and of the altered conditions produced by the operation might perhaps have caused their production in extraordinary numbers soon after it took place and before six and a half months had elapsed there might have arisen in this way a sufficient number of new cells to form a complete epithelial lining for the gland bodies and the process may thereupon have ceased. It is also possible that the mucous cells of similar character in the neck region, which are seen frequently undergoing mitotic division, might have produced many new cells which moved down into the gland bodies and replaced the ferment cells which were disappearing. His assumption that the new mucous cells arise by transformation of zymo-

genic cells does not therefore seem necessary to account for the phenomena he observed, and it would require for its establishment the study of stages other than that of six and a half months after operation. Fifth: Do changes of a reverse nature go on in the pyloric region of the stomach when the pylorus is occluded? In the light of Cade's conclusion, one might expect such changes to occur, when the mucous membrane of this region is placed in a position where it is made part of the fundus and no longer performs its usual functions. This can be effected by artificial occlusion of the normal pylorus, and the establishment of a new one by gastroenterostomy in another part of the stomach. The development in the pyloric glands under these conditions of changes, which would tend to make them resemble more closely the fundus gland, would seem the more probable, since occasional fundus elements have been found in the pyloric mucosa. Stöhr, 82, found parietal cells in human pyloric glands. They have been observed by Cade, 01, and Renaut (reported by Cade) in human pylori partially occluded by carcinoma. In man Bensley, 03, has found them beyond the pylorus in the glands of Brunner.

It was obvious that these questions could be answered only by the study of postoperative changes in both regions of the stomach at many stages, and in view of the importance and significance of the conclusion reached, and the interesting nature of many of the questions arising, it seemed worth while to extend experiments along this line and to study with the newer technical methods these changes at many stages.

Personal Experiments.

I performed gastroenterostomies on a large number of dogs, and in those which were to be kept one month or longer I occluded the pylorus also. At the time of operating, several small pieces of mucosa were removed from the area involved and fixed in various solutions, with a view to using them as controls with which the mucous membrane modified as a result of the experiments might be compared later. They served also for the study of the structure of the normal, healthy, gastric mucous membrane. The gastroenterostomies were performed on the anterior surface of the stomach by Woelfler's[1] method of suture only, and there was little trouble in getting satisfactory results. One or two made on the posterior surface by Von Hacker's[1] method were equally satisfactory. Operations on the anterior surface in the dog produce a new pylorus in a part of the stomach which is dependent when the dog is on his feet,

[1]Described in Bryant's Operative Surgery, 05.

thus facilitating the passage of food into the duodenum. The anastomosis was 4 to 5 cm. long and was distant from the pylorus at least 12 cm. on the stomach, and 20 cm. on the duodenum. It is advisable that the gastric opening be distant at least 10 cm. from the normal pylorus, in a dog of medium size, in order that it may be undoubtedly in the fundus region. In one animal one end of the anastomosis was intentionally disposed in the pyloric region about 6 cm. from the pyloric valve.

More trouble was experienced in occluding the pylorus. After ligature with heavy silk its lumen was re-established in a month. Then the pylorus was plicated, so as to produce a longitudinal fold on its ventral surface. The edges of this fold were united by a few fine silk sutures, and two ligatures of heavy silk were tied around the whole about 1 cm. apart. After 10 months the lumen was partially established. The following method finally adopted was always successful. A longitudinal incision 2 to 3 cm. long was made on the ventral and another on the dorsal surface of the pylorus. They extended through the serous and muscular coats. The mucous tube was then freed and cut across. The ends were closed with fine silk and pushed into the stomach and duodenum. The edges of the wound were then pulled out laterally so that, while originally longitudinal, it became transverse. The edge in front of the stomach was then sutured firmly with heavy silk to that behind it, and the same procedure followed with the anterior and posterior duodenal edges. It is necessary that these sutures be made with heavy silk and that a large piece of tissue be included, because of the powerful contraction of the m. sphincter pylori. By this method the continuity of the vascular and nervous structures at the margins of the pylorus are preserved.

The dogs were killed at stages after the operation of 2, 4, 7, 9, 12, 14, and 15 days, and of 1, 2, 3, 4, 5½, 6½, and 10 months. The stomach was opened and the condition of the anastomosis and pylorus noted. The area of the anastomosis selected for study was that farthest removed from the pylorus in order to be sure to avoid the intermediate region. In one case which will be especially described later, material was taken from the pyloric end of the anastomosis also. This was the case in which one end of the anastomosis was actually in the pyloric region. For the study of the results following occlusion of the pylorus the material was selected from the center of the pyloric area. The changes occurring in the duodenum were not especially studied. Search was made, how-

ever, in nine or ten cases for ulcers in the part of the duodenum proximalward from the anastomosis, where they have been reported after similar operations on the human subject. They did not appear in my dogs.

RESULTS.

1. *Degeneration.*—Examination of sections shows that immediately after the operation there is degeneration of the glands which are struck by the knife or included in a suture. This process is limited to a very few glands which lie next the line of operation. Not more than three or four are affected. Sometimes the glandular elements undergo degeneration *in situ* but frequently are cast off, some of them preserving the structure characterizing them in the healthy mucous membrane, but most of them showing evidence of degeneration. In preparations of two and four day stages there are constantly found in immediate proximity to the lines of incision, masses of débris which give evidence of their derivation from all forms of glandular elements. They occupy the lumina of glands or lie free on the surface of the mucosa. Occasionally they contain intact cells retaining their nuclei and cytoplasmic structure, but these are not numerous and for the most part the cell contents have no enclosing membrane or definite outline, and those of one cell are not marked off in any way from those of neighboring cells, but all form a common mass in which elements of various cells are found distributed, retaining their power of acting specifically with stains. Zymogen granules, usually extremely coarse, and resulting perhaps from the confluence of smaller ones, appear in neutral gentian preparations in considerable numbers, and copper-chrome-hæmatoxylin preparations show many characteristic parietal cell granules, while in many places the substance between the granules shows a positive reaction with muci-carmine.

The body chief cells are the first of the glandular elements to show evidence of degeneration. Their nuclei become swollen and pale, and poor in chromatic material, each containing usually a single small mass of chromatin, although a few nuclei contain several small chromatic bodies. In a few the nuclear membrane is broken down and the karyoplasm distributed throughout the cell. The cytoplasm usually contains a few large zymogen granules but no basal filaments or organized prozymogen. As Garnier, 97, has pointed out, prozymogen is probably complex and unstable, and in cells injured by the operation, whatever of it is present at the time is probably at once transformed into zymogen granules and no more is produced. The cytoplasm of most cells contains also many vacuoles, and in all cells it is reduced in amount.

The parietal cells are more resistant than the chief cells and con-

sequently appear relatively more numerous in areas of degeneration than in the healthy mucous membrane. This relative stability of the parietal cells and their resistance to destructive influences has been noted in the human subject by many observers. Böckelman, 02, Hammarschlag, Korcynsky and Jaworsky, 02, and Bouveret, 93, mention it in gastric ulcer. Popoff, 97, states that it is every evident in inflammatory conditions of the gastric mucous membrane in the dog. Their stability was very evident in my preparations, as parts of the two or three degenerating glands were composed almost exclusively of parietal cells, all the other glandular elements having degenerated and disappeared. Many of the parietal cells remaining retained a full and definite outline and appeared little affected by degeneration. Many others of them, however, lost their regularity of outline, becoming oblong, square, or pyriform in sections and presenting changes in both the nucleus and cytoplasm. The former, while partaking sometimes in the irregularity of the cell outline, are usually spherical, being pale and swollen and containing two or three small masses of chromatin. Occasionally the karyoplasm takes a diffuse and homogeneous nuclear stain, showing that the nucleus is undergoing degeneration by karyolysis. In nearly all of them, however, the nuclei degenerate by karyorhexis, although the pyknotic stage which is described by Schmaus and Albrecht, 95, as developing at the termination of this process and in which the chromatic material is massed into a ball, does not appear. The earlier stages, however, were common in which the chromatin disappears from the interior of the nucleus and lies upon the nuclear membrane, which later becomes broken down and the nuclear material distributed throughout the cell. The cytoplasm commences to degenerate earlier than the nuclei. It becomes reduced in amount and its granules become fewer and paler. Commonly a circular area appears about the nucleus, from which the granules have either disappeared completely or become unstainable. This area appears absolutely empty and often it extends throughout the whole of the cytoplasmic part of the cell.

A feature of these degenerative processes is the resistance of the nuclei. Although this appears sometimes in the chief cells it is much more marked in the parietal cells, where the nucleus usually persists pale and swollen, but entire, after most of the cytoplasm has disappeared. They often form masses in which the individual nuclei are separated by only a very little cytoplasm which, retaining its distinctive character, determines the nature of the cell. This same nuclear stability is reported in the parietal cells of the human stomach in cases of ulcer, where Böckelman, 02, found that sometimes the cytoplasm had so completely

disappeared that it was very difficult to distinguish such parietal cells from lymphocytes. The same difficulty has presented itself in my work.

Another feature is the infiltration of the interglandular connective tissue by small round cells and the very great increase in the number of red blood cells in the capillary vessels. The latter is probably due to inflammatory venous stagnation. It is most marked in the region of the gland necks and the deeper parts of the foveolæ, where the vessels are always greatly distended and where there are sometimes extravasations.

The degenerative process goes on during a few days following the operation. At stages of two and four days it is very marked, being less evident in later stages until in fourteen day stages it hardly appears at all. It is always limited to three or four glands at the most, lying in immediate proximity to the line of incision and suture. The loss of these glands by degeneration does not as a rule leave a gap in the mucous membrane, because the redundancy of the gastric fundus mucosa tends to keep the margin of the undegenerated portion opposed to undegenerated duodenal mucosa, with which it becomes continuous.

2. *Changes in gland lumina and foveolæ.*—In the glands surrounding the anastomosis, which are not injured by the knife or sutures, no acute or extensive degenerative processes like those described above go on. There are, nevertheless, very considerable postoperative changes. The foveolæ and gland lumina become dilated to form cystlike structures. These appear at first within two or three days after the operation in in the bottoms of the foveolæ only, but by the fourth day appear also in the gland necks and bodies. They are formed first in the glands next the line of operation and in later stages involve other glands as well, being found progressively farther away from it up to two week stages, when they exist throughout a zone 1 cm. in width surrounding the anastomosis and including over one hundred glands. In early stages these dilatations appear as spherical or oval structures distended with secretion. A little later they are often pear-shaped, and at two weeks' stages they are usually more or less completely collapsed and confined for the most part to gland bodies, appearing in every fifth or sixth gland only. They usually form disc-like structures at the end of the gland lying next the muscularis mucosæ, between it and the ends of other glands not so enlarged. After two weeks they begin to disappear, first from the glands farthest from the anastomosis, and by three months they are only occasionally present. One very large closed cyst appeared at the line of union in one of my six and a half months' preparations. It was approximately spherical, and extended throughout the thickness

of the mucosa. In nearly all preparations of late stages small cysts appeared occasionally. In sections of material taken from the pyloric end of the anastomosis, which extended into the pyloric region, and studied five and a half months after operation dilatations in the gland lumina appeared throughout the section. A section from the cardiac end of the same anastomosis was clearly in the fundus region and showed cystic dilatations in three glands only next the line of union of gastric and intestinal mucous membrane. Similar cystic structures appeared also in the duodenum. Such cystic dilatations appear constantly in considerable numbers in the healthy mucous membrane of the cardiac region (Schaffer, 97, and Bensley, 02), but in the fundus region they seem to be associated always with subacute or chronic inflammation. In my preparations they occur in a place where the mucous membrane has been subjected by the operation to a very considerable irritation which is perpetuated by the abnormal conditions introduced by the operation. Such cystic dilatations appear in gastric ulcer (Krukenberg, 88) and some dilatation of gland lumina has been reported in ulcer, carcinoma, and chronic gastritis by nearly every pathologist who has written upon these subjects. Occasionally they are filled with a homogeneous, or sometimes (in alcohol hardened material), granular substance which, because it stains in mucus staining dyes I consider to be mucus. Frequently they are empty or contain only a very small quantity of mucus as a layer upon their walls. Their primary formation probably depends upon the increase in the secretion of mucus which goes on during most inflammations of the stomach, and probably also on an associated increase in its tenacity interfering with its rapid removal and producing accumulations in these dilatations. The fact that some are empty may be explained by the digestion in situ of the mucus and its replacement by a watery substance, which does not remain in the section. Cade, 01, points out that by this dilatation of their lumini the glands approximate the pyloric type, and makes of this point a confirmation of his conclusion that the glands near the anastomosis are changing in nature and becoming true pyloric glands, but since such a change occurs generally in gastric inflammations, and since an inflammatory process exists in the glands under consideration, that seems to afford a reasonable and sufficient explanation of the dilatation.

In the cells of the foveolar wall there are no marked post-operative changes. Where the foveolæ are greatly distended the cells become shorter and may be cubical or even flattened with corresponding alterations in the form of the nuclei which lie in their attached ends. Otherwise these cells retain their normal characteristics.

3. *Changes in the parietal cells.*—The parietal cells, which Cade did not find in the glands next the anastomosis, appear in all my preparations, extending quite to the line of union. Up to stages two months after operation they are sometimes, though not always, less numerous than normally, in the two or three glands nearest the line of union. At stages of three months and later they appear in nearly or quite normal frequency in all the glands of the section, including those near the anastomosis. But while their number does not appear materially altered, some of them present changes after the operation, which may be attributed to it.

In many glands there seems to be more variation than normally in the size of these cells. Four months after operation most of the parietal cells in glands near the anastomosis are smaller than in glands farther away. This I thought due possibly to pressure atrophy, since in this preparation the gastric mucosa was overlapped for a short distance by the duodenal. As in many other cellular structures affected by inflammation there is among the parietal cells a tendency to hyperplasia. Some few become quite large, possessing a diameter two or three times as great as that of ordinary parietal cells and provided with multiple nuclei, three appearing sometimes in my preparations. They are rich in granules taking the characteristic stain. They often contain vacuoles and frequently a very large number of spirilla (Fig. 1). They are found usually near the muscularis mucosæ and exist long after the operation, being found in preparations of ten months' stages. Such large parietal cells are described by Böckelman, 02, in gastric ulcer in man attaining sometimes a diameter of thirty micra and containing two to four nuclei.

The yellowish-green cells described above, which show an affinity for chromic acid salts, appear in somewhat larger numbers than in the normal mucosa, particularly during the week after operation, when nearly every gland section contains three or four cells of this type. Although most frequent in the gland bodies they are occasionally present in the necks. They are often more irregular in outline than the neighboring parietal cells. Their nuclei are usually spherical and poor in chromatin. In the two or three glands struck by the knife which undergo degeneration they are not more frequent than in glands at some litlte distance from the incision. But from their irregular shape and their increased frequency in areas of subacute inflammation they would seem to be degenerative forms.

In preparations six and a half months after operation I found in the parietal cells of gland necks close to the anastomosis a very distinct, cloudy area, near the nucleus (Fig. 4). Its outline was usually irregular

and it occupied from one-third to two-thirds, or even more, of the cytoplasmic area of the cell. It seemed nearly always closely associated with the nucleus and was frequently applied about it in a crescentic manner. It stained purple with neutral gentian in which stain it is very definitely and distinctly marked, but was not clearly demonstrable by any other method. Every parietal cell in the neck region contained it, but it was present in only a few cells of the gland bodies. The nature of this substance is in doubt, but its appearance does not at all suggest that it is constituted by the remains of broken down nuclei, which Cade describes as existing in these cells.

In the walls of cystlike dilatations many parietal cells are found flattened and extended, constituting part of the cyst wall and lying next the lumen, between chief cells. They have flattened nuclei and are somewhat poorer in granules than normal cells, those present, however, showing clearly the staining reactions of characteristic parietal cell granules.

In a dog in which a small stomach pouch had been isolated from the fundus region and made to communicate with the surface by the Khigine-Pawlow operation, Cade, 03, examined the glands at the surface opening of the fistula at a stage six and a half months after the operation. He reports that parietal cells, while absent from a few glands, were found in most of them, and while he believed that those which he found were "in the way of disappearing," their presence in that situation seems to be in accord with my findings in the neighborhood of a gastroenteric anastomosis. He was not able to find them, however, in the neighborhood of the gastroenteric anastomosis, which he made in a dog and examined at the same stage after operation, a result which seems surprising since if they exist in fundus glands at the surface of the body, where they must be subjected to great irritation, it would seem unlikely that they would disappear from an internal part of the stomach where the irritation must be much less. This result seems also difficult to understand in view of the fact that I have found them constantly in my preparations.

4. *Changes in the neck chief cells.*—The neck chief cells, like the foveolar cells, are sometimes flattened by reason of the enlargement of the lumina. A few of them contain vacuoles in their cytoplasm. Otherwise they preserve their normal structure.

5. *Changes in the body chief cells.*—The body chief cells show very striking changes in the neighborhood of the anastomosis. Immediately after the operation the cytoplasm becomes vacuolated. This was especially marked at four days after the operation, but in the stomach from which my preparations of this stage were made the wound margins did

not appear healthy. In other stages soon after the operation vacuolization, although present in the chief cells, was not nearly so well developed.

The ferment forming function of these cells is completely lost. Absolutely no zymogen granules can be demonstrated in their cytoplasm by the neutral gentian or any other stain which I have employed. They assume, however, the appearance and properties of mucus forming cells, and contain in their free ends masses of substance staining in mucicarmine and muchæmatein. There is a transformation of ferment-forming cells into mucus-forming cells. The change is a gradual one, commencing within the first week at the line of anastomosis and extending radially during the following two or three weeks into the mucous membrane around it. It reaches its widest extension at some time between two weeks and a month after the operation and at the end of two weeks no zymogen granules whatever can be found within a zone 7 mm. wide surrounding the line of union and including within it fifty or fifty-five gland bodies to the radial section. In these glands the bodies are formed, in addition to parietal cells, of chief cells containing mucus and differing in no demonstrable respect from the neck chief cells (Fig. 2).

Outside this zone the chief cells of the gland bodies contain zymogen granules, but in the cells of glands immediately outside it, lying at a distance of about 7.5 mm. from the line of union, they are present in much smaller numbers than normally appear. They gradually increase in number, however, as one studies glands successively farther removed from the line of union. The first gland in which they appear contains often only one or two ferment cells and these contain only a few granules in each. Beyond this gland the number of ferment cells and granules steadily increases until the body chief cells are packed with granules between the nucleus and the free end and contain prozymogen between the nucleus and the attached end. It must be borne in mind, however, that in the normal mucous membrane there are found among the body chief cells occasional mucous cells between the ferment cells.

At the end of one month after operation the ferment forming function of the modified body chief cells farthest away from the line of union commences to reassert itself and a few zymogen granules begin to appear in them again. This process extends gradually toward the anastomosis until, at six and a half months after the operation, the chief cells of the gland bodies next in line of union contain zymogen granules in quite normal numbers and do not differ in any respect from the corresponding cells in parts of the fundus region remote from the site of operation. While this process is going on it may sometimes be observed that the ferment cells nearest the line of union are somewhat distant from the other

ferment cells and appear as small islands in an area in which all the other cells contain mucus. This occurred in my preparations of three and four month stages.

The exact extent of this change at the various stages after the operation is shown in the following table:

At 7 days it extends through 6 glands, with very few ferment cells in the next 40 glands.
At 9 days it extends through 8 glands.
At 12 days it extends through 22 glands.
At 15 days it extends through 55 glands.
At 1 month it extends through 50 glands.
At 2 months it extends through 36 glands.

At 3 months it extends through 30 glands, except for two glands near the anastomosis, which contain three or four ferment cells in the ends of each.

At 4 months it extends through 18 glands.
At 5 months it extends through 4 glands.
At 6½ months it extends through 3 glands. (In one case.)
At 6½ months it extends through no glands. (In a second case.)
At 10 months it extends through no glands.

In the last two cases ferment cells were found in glands next to the line of union.

This table shows the extent to which zymogen granules are entirely absent from body chief cells. This extent can be easily determined, since there is no difficulty in detecting these granules in neutral gentian preparations even when only one or two are present in the cell. The intermediate zone immediately outside it, across which the number of granules gradually increases till the normal is attained, is wide enough to include ten to thirty glands to the radial section.

Just as in the loss of the zymogenic function the prozymogen disappears before the zymogen granules, so in the reassumption by these cells of their zymogenic function, the prozymogen appears in the basal part of the cell before zymogen granules can be demonstrated in the free end, and in advance of those glands in whose cells zymogen granules appear are found glands whose cells, while containing in the free ends a substance showing an affinity for mucus staining dyes, contain in their attached ends a substance showing an affinity for toluidine blue and which resembles prozymogen. Such cells occasionally form the walls of small cystlike dilitations relatively remote from the line of union.

There is, therefore, during the first two or three weeks after the operation, in the bodies of many glands immediately around the anastomosis a gradual replacement of the ferment cells by mucous cells, extend-

ing peripheralward, and a reversal of this process during the following five or six months, during which there is a replacement of these mucous cells by ferment cells, extending centralward. The same phenomena followed the suturing of simple incisions which I made through the gastric mucosa in the fundus region. Throughout all stages of these processes the increase in number of one kind of cell is exactly proportional to the decrease in number of the other kind, a fact which suggests strongly that the new mucous cells formed in the first month and the new ferment cells formed in the following months arise each by transformation of previously existing cells of the other variety. This is confirmed by the fact already noted that when the process of ferment formation in cells is extending toward the anastomosis during the four or five months following the one immediately after the operation, the most advanced ferment cells, that is those nearest the line of union, contain only a very few zymogen granules. During the first month, too, while the mucus forming process is extending into gland bodies farther and farther from the anastomosis, the most advanced mucous cells contain only a little mucus, which stains feebly, while those near the line of union are almost full of mucus and take a very strong stain with mucicarmine.

A consideration of the other possible modes of derivation of these cells, namely, division of previously existing cells of the same kind, or transformation of parietal cells, shows that they do not arise in either of these ways. There are no indications that the new mucous cells which are formed within a month after the operation arise by division of previously existing mucous cells in the gland bodies. Mitotic divisions among these cells are extremely rare and there is no appreciable increase in the number of these mitoses after the operation. It is also very unlikely that many of them arise by proliferation of the mucous chief cells of the gland necks and travel down the glands to replace the ferment cells of the bodies, because there is very little, if any, increase in the number of mitoses in the neck region and there is no evidence of active migration of cells at this time. Further, there are no evidences of degeneration or disappearance of body chief cells except in the two or three glands which degenerate on account of injury by the knife or sutures. The new ferment cells which appear in gland bodies between the first and seventh months after operation cannot arise by transformation of previously existing ferment cells, because examination of preceding stages shows that absolutely no ferment cells existed in any part of the glands in the situation where new ferment cells arise. The transformation of parietal cells into chief cells, while suggested by Trinkler,

83, and Pilliet, 87, has not been confirmed by other observers. Bensley has not been able to find it and in my preparations I have found absolutely no evidence of their transformation into chief cells of either variety. Further, while new mucous or ferment cells are being formed, the parietal cells of the gland bodies are not diminished in number nor are there mitoses among them, so that new chief cells cannot arise from them. There remain only the ferment cells from which new mucous cells can be derived, and only the mucous cells from which new ferment cells can be derived, and so by a process of elimination of every other source possible, there is afforded a very strong confirmation of the indication that cells of these two varieties are transformed one into the other.

Stages of the transformation may be actually observed, in which the same cell contains both mucus and zymogen. In a preparation of the stage of two weeks after the operation I stained a section with neutral gentian and selected in it a gland containing cells with only a few zymogen granules in them. Between this gland and the line of operation all gland bodies contained chief cells which were exclusively mucus-forming. A few cells of this gland were drawn showing zymogen granules in their free ends. The section was then put in alcohol until all the neutral gentian was extracted and then stained in muchæmatein. The same cells were then found to contain considerable numbers of mucous granules.

From all these facts it seems necessary to conclude that ferment cells may be transformed into mucous cells, and that these same cells may later lose their mucus-forming function and reassume the morphological characters and function of ferment-forming cells, which they possessed before the operation.

The replacement of ferment cells by mucous cells was observed by Cade, 01, who assumed that it was a transformation and interpreted it as evidence of the formation of a new pylorus, and he concluded therefrom that the body chief cells are not specifically set apart for the performance of one function, but that they possess a morphological flexibility and take on a pyloric character when made to perform the part of pylorus. In view of the fact that in addition to the alteration of function caused by the establishment of the new pylorus, there is affecting these cells, also an inflammation produced by the mechanical violence of the operation and by the tension which the attachment to the duodenum makes upon this area of mucous membrane, it seems to me that this explanation is incomplete, since it takes account of only one of the two influences which are operating to produce changes in the cells. The change might be merely the expression of the cellular reaction to

the inflammatory process, which might disappear upon the termination of the inflammation. Since these cells afterward resume their former characters and function in spite of the persistence of the new pylorus, it seems necessary to conclude that they undergo a mucous transformation simply because they are in an inflammatory condition, and not because they play the part of pylorus and therefore, in response to a great biological law which requires the morphological adaptation of structures to correspond to the performance of new functions, undergo a transformation making them like pyloric cells. Such a mucous transformation is common in inflammatory conditions in the stomach. Meyer, 89; Hayem, 92; Ewald, 93; Boas, 94; Schmidt, 95; Popoff, 97, and many others report it as occurring in gastritis. Sachs, 87; Schmidt, 96; Leuk, 99, and many others report it in ulcer. Böckelmann, 02; Lubarsch, and others have described it in carcinoma. Cade, 03, has described it at the surface opening of a fistula leading into a stomach pouch isolated from the general gastric cavity of a dog.

Most pathologists regard this mucous transformation as a retrograde change, and it is frequently referred to as a mucous degeneration. Letulle, 00, speaks of it as a "probably irreparable" change. Cade believed it to be permanent. He observed it six and a half months after the operation of gastroenterostomy in the dog and laments the lack of preparations of later stages, in which he expected to find the changes much more pronounced. I made two preparations of the six and a half months stage, in one of which the ferment cells extended to within three glands of the anastomosis, and in the other they extended quite up to it. In the ten months' preparation they extended quite to the anastomosis, and the glands containing them, although lying next to duodenal glands and not separated from them by more connective tissue than is usually found between adjacent gastric glands, nevertheless did not differ in any essential particular from normal gastric glands in the fundus region. (Fig. 3.) Cade, 03, reports also certain other observations which he made of conditions in which this mucous transformation of ferment cells was found persistent for long periods. In the experiment mentioned above, in which a fistula was made in a dog, putting a stomach pouch made in the fundus region in communication with the surface of the body, he found, six and a half months after the operation, that at the orifice of the fistula the chief cells of the bodies of the glands had become transformed so as to resemble closely the chief cells of the gland necks or of the pyloric glands. The mucous membrane of the pouch walls generally was not modified but preserved the character normally found in gastric mucosa of the fundus region.

Cade and Latarjet, 05, observed also a case in which these transformations of ferment cells into mucous cells persisted for many years. An infant had had a gastric hernia which opened to the surface spontaneously during the first year of its life. The part of the stomach with which it communicated was later separated from the general cavity by strangulation of the hernia and constituted a separate gastric pouch. About the margins of the fistula by which this pouch communicated with the surface, the mucous membrane was found, after nineteen years, to contain glands derived clearly from fundus glands but containing large lumina and formed of a single layer of rather cubical cells which were in all essential points like pyloric gland cells. The walls of the small stomach were formed of normal fundus mucosa. In this case the ferment forming chief cells of the gland bodies had been replaced by mucous cells probably during the first year of life, and the mucous character had been preserved for nineteen years. But in both these cases of gastric fistula the conditions present are not those existing at a new pylorus formed by gastroenterostomy. The opening to the surface subjects the glands near it to irritation from garments, bacteria, friction, etc., and to excoriation from drying combined with friction, all of which combine to produce a continued irritation which must tend to keep the glands affected in a condition of chronic inflammation, and so perpetuate in them phenomena which we have seen to be characteristic of inflammatory conditions.

In view of the subsequent reassumption by these cells, in my experiments, of their original function, after performing another function for several months, it seems necessary to believe that in the dog's stomach the mucous transformation is not necessarily an irreparable, retrogressive change.

And since such transformations are only temporary after simple incisions and after gastroenterostomies, it seems quite justifiable to conclude that they may be only temporary after inflammations from other causes also, that they are not necessarily degenerations nor even final differentiations, but are merely a passing stage of the cytomorphosis of these cells into which it is quite within their capacity to pass for months, and from which they may return to the assumption of their former characters and the performance of their former function. It should, therefore, be possible that in stomachs in which chronic inflammation has resulted in the change of ferment cells into mucous cells there may occur a changing back again of these same cells into ferment cells and a resumption of their original activity. This may serve to

explain to some extent certain clinical phenomena, for example, those reported by Einhorn, 02, in two patients in whose stomachs he found the ferment cells transformed into mucous cells but whose health and digestion were comparatively good two years later.

The transformation of mucous cells into ferment cells has not been commonly observed. Cade, 01, suggested the possibility of such transitions but says the facts are not enough to enable one to affirm them. Bensley and Zimmermann considered the two forms of cells specifically distinct, and therefore transitions from one form to the other and back again were not expected *a priori* by the writer.

6. *Results of occlusion of the pylorus.*—After occlusion of the pylorus so that no food passes through it, a new pylorus having been formed by gastroenterostomy in another part of the stomach, I was not able to find any changes whatever indicative of the assumption by the mucosa in this region of the characters present normally in the fundus region. There was sime infiltration by round cells, but there was not at any stage up to and including that of ten months after the operation, any transformation or replacement of pyloric gland cells by ferment cells. No zymogen granules or prozymogen in any form appeared in the cells, nor could I find any parietal cells. The glands were always typical pyloric glands, in the relative depth and breadth of foveolæ and of gland lumina, in the general character of the glands and in the structure of the glandular elements constituting them. When, therefore, the conditions of existence of the pyloric mucous membrane are altered and it is placed as far as possible in the position normally occupied by fundus mucosa, it does not show any tendency to assume the morphological characteristics of the fundus mucosa. It must be borne in mind, however, that in this experiment the gastric musculature is unaltered and, it may be presumed, acts in exactly the same way as before the operation. So that while no food actually passes the pylorus, the mucous membrane there is still subjected to its mechanical action to a very considerable extent, as the muscles of the stomach wall force the contents along the old course against the pyloric mucosa.

7. *Significance of results observed.*—The retention by pyloric gland cells of their original characters after occlusion of the pylorus, and the complete return of the cells of the glands near a new pylorus to their original form and function even after a transformation of over six and a half months duration, and in spite of their subjection to the action of external conditions, which are very considerably changed, seems to indicate for these cells a certain specificity. It does not seem to support

in full the contentions of Bard, 98, who believes that there is inherent in the cell by its heredity a predestination to a certain form and function unalterable by the action of external conditions except within the narrowest limits. But it indicates that cells, which from whatever cause, either inherent or external, have reached a certain stage of differentiation and specialization, do not possess the power to respond readily to the action of external influences. They tend to retain the form and function which they have attained, even when subjected to the action of external conditions very different from those which have previously been acting upon them. The ferment cells, however, though very highly specialized, frequently in response to changed conditions assume a form through which they have probably passed during the development of their special characters. This retrograde step is not necessarily a degeneration or final differentiation, for they possess still a tendency on the removal of the disturbing conditions to become again specialized. And this further development tends to proceed by lines along which the cell has previously travelled in its cytomorphosis. To this extent my observations show that such highly differentiated cells possess a kind of specificity. But the tendency to develop along such lines is not an imperative necessity to the cell. It retains the power of developing along other lines also, if certain other external conditions be present, and the number of transformations presented by gastric chief cells is very considerable. Schmidt, 96, reports that he found these cells changing into intestinal epithelium consisting of goblet cells and cylindrical cells with a striated cuticle, at the margins of ulcer and in chronic inflammation. Leuk, 99, found goblet cells among them. Hári, 01, reports them in both healthy and pathological conditions in various parts of the human stomach, often in the fundus region. Schaffer also reports the existence in the human stomach of similar patches of intestinal epithelium which he regards as elements dislocated from the duodenum. But the fact that he found them in the part of the stomach farthest removed from the duodenum, coupled with their absence from a healthy human stomach taken from an executed man and examined by Bensley and Revell, and with the fact that they have not been observed in stomachs of other mammals, notwithstanding the very great number which have been examined, but usually in a healthy condition, seems to justify the conclusion that they are not dislocated duodenal epithelium but are transformations of gastric epithelium under the influence of pathological conditions. Dean D. Lewis has shown me such intestinal epithelium taken from the fundus region of a human stomach which was affected with carcinoma. Ham-

merschlag, 96; Cohnheim, 96; Böckelman, 02, and others report similar observations. Lubarsch has seen it in cases of achylia gastrica. Ewald, 93, and Meyer, 89, have illustrated it as occurring in chronic gastritis.

Such transformation seems to a certain extent in accord with the conclusions of O. Hertwig, 98, according to whom cells of various kinds inherit equivalent potentialities, many of which they appear to lose gradually during the successive stages of their cytomorphosis, while assuming more completely and exclusively the performance of special functions, but retaining throughout a large part of their life the ability to assume forms and functions quite different from those ordinarily associated with them, when subjected to the influence of varying external conditions. And while it seems quite possible that cells may attain so high a degree of specialization as to lose completely the power of undergoing any further transformations, except degenerative ones, it must be borne in mind that, unfortunately, we possess no criterion by which this condition can be definitely determined. And since, as in the case of the ferment forming grandular elements of the dog's stomach, many cells which appear to perform one very highly specialized function, and under normal conditions, present invariably associated morphological characteristics, may nevertheless retain the power of performing actively very different functions after undergoing great transformations, it is not possible to conclude that any cells are specific before subjecting them to prolonged study by experimental methods.

In conclusion, it is a pleasure to express my gratitude to Prof. Bensley, under whose direction this work was done.

SUMMARY.

Cells possessing a special affinity for chromic acid salts and resembling closely the chromaffine cells, described by Kohn in the suprarenal gland and elsewhere, exist normally among the parietal cells of the dog's stomach.

After gastroenterostomy the mucous membrane within 7 mm. of the line of operation undergoes the following changes.

The body chief cells, which are normally ferment-forming, become transformed into mucous-forming cells. This is a gradual process beginning immediately after the operation, at the line of suture, and extending radially about the anastomosis. It reaches its maximum extent of 7 mm. about three weeks after operation. Ferment cells in the next 3 mm. show a tendency to this change, but do not undergo a complete transformation. After one month a reverse transformation commences,

and the same cells again become ferment cells. This process is completed by six and a half months after operation. After that the gastric glands next to duodenal glands at the anastomosis do not differ materially from those remote from it. This is a transformation of cells, not a replacement. Cells were found containing both mucus and zymogen.

Many parietal cells enlarge; many become vacuolated. The number of cells with an affinity for chromic acid salts is considerably increased, especially within a week after the operation.

When a new pylorus was formed in the fundus region of the stomach by gastroenterostomy, the glands around it did not show any permanent changes indicative of their assumption of the character of pyloric glands.

When the pylorus was occluded also, the pyloric glands did not show any tendency to assume the characters of fundus glands.

The gastric glands tend to retain their normal characters even under the influence of changed external conditions. If they undergo transformations in response to the action of such changed conditions, they tend later to resume their original characters. But they do not always do so. Metaplasia of gastric glandular epithelium has been observed to a considerable extent in various conditions, mostly pathological.

Since cells very highly specialized may undergo such transformations, the term "specific" must be applied to them with care, and only after prolonged study by the experimental method.

AUTHORS CONSULTED.

Bard, L., 98.—La spécificite cellulaire, ses conséquences en Biologie générale. Scientia, Série Biologique. I. Evereux, Imprimerie de Charles Hérissey.

Bensley, R. R., 96.—The Histology and Physiology of the Gastric Glands. Proc. Canad. Inst. Toronto, I, 11-16.

——— 98.—The Structure of the Mammalian Gastric Glands. Quart. J. Micr. Sc., Lond., N. S., XLI, 361-89.

——— 00.—The Œsophageal Glands of Urodela, Biol. Bull., Boston, II, No. 3, 87-104.

——— 02.—The Cardiac Glands of Mammals, Am. J. Anat., Baltimore, II, 105-56.

——— 03.—The Structure of the Glands of Brunner. The Decennial Publications of the University of Chicago, X, 279-326.

——— 04.—Stomach. Reference Handbook of Medical Science, VII, Wm. Wood & Co., N. Y.

Bizzozero, G., 93.—Ueber die Schlauchförmigen Drüsen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflächenepithel der Schleimhaut. Arch. f. mikr. Anat., Bonn, XLII, 82-152.

Boas, I., 94.—Diagnostik und Therapie der Magenkrankheiten, 3 Aufl., Leipz.

Boeckelman, W. A., 02.—Untersuchungen zur pathologischen Anatomie des menschlichen Magens in Fallen von Ulcus und Carcinoma bei bekannter chemischen und motoischen Funktion. Ztsch. f. klin. Med., Berl., XLIV, 128 et seq.

Bonnet, R., 93.—Ueber den feineren Bau der Magenschleimhaut des Menschen und einiger Hausthiere. Deutsche Med Wchnschr. Jahrg. 19, No. 18, 430-1.

Bouveret, L., 93.—Traité des maladies de l'estomac, J. B. Baillière et fils.

Bryant, J. D., 05.—Operative Surgery. II, 905-908, New York, D. Appleton & Co.

Cade, A., 01.—Etude de la constitution histologique normale et de quelque variations fonctionnelles et expérimentales des éléments sécréteures des glandes gastriques du fond chez les mammifères. Arch. d'anat. micr., Par., IV, 1-86.

Cade, A. et Latarjet, A., 05.—Realization pathologique du petit estomac de Pawlow. Etude physiologique et histologique. J. de physiol. et de path. gen., March. 221-233.

Cobelli, R., 65.—Le ghiandole acinose della parte pilorica dello Stomaco. Sitzungsb. k. Akad. d. Wissensch., Math. naturw. Col., L, 1 Abt., Wien, 483.

Cohnheim, P., 96.—Die Bedeutung kleiner Schleimhautstückchen für die Diagnostik der Magenkrankheiten. Arch. f. Vrdngskrankh. I, 274-298.

Deimleb, K. M., 04.—Vergleichende Untersuchungen über die Pylorusdrüsenzone des Magens und die Duodenal drüsenzone des Darmkanals der Haussäugetiere. Inaugural Dissertation zur Erlangung der philosophischen Doktorwürde. Zürich.

De Witt, Lydia M., 03.—On the Morphology of the Pyloric Glands of Vertebrates. Cntrb. Med. Rsrch, Vaughan, Ann Arbor, Michigan, 188-203.

Ebstein, W., 70.—Beiträge zur Lehre vom Bau und den physiologischen Functionen der sog. Magenschleimdrüsen. Arch. f. mikr. Anat., Bonn, VI, Heft. 4, 515.

Edelmann, R., 89.—Vergleichend-anatomische und physiologische Untersuchungen über eine besondere Region der Mageuschleimhaut (Cardialdrüsenregion) beim den Säugetieren. Deutsche Ztsch. f. Thiermed. XV, Leipz., 165-214.

Einhorn, M., 02.—A further Contribution to our Knowledge of the Histology of the gastric Mucosa in pathological Conditions of this Organ. Am. J. M. Sci., Phila., CXXIV, 571-589.

Ewald, C. A., 93.—Klinik der Verdauungskrankheiten. II., 3 Aufl., Berlin.

Garnier, C., 97.—Les filaments basaux des cellules glandulaires. Bibliog. anat.

Golgi, C., 93. Sur le fine organisation des glandes peptiques des Mammifères. Arch. ital. de biol., Turin, XIX, 448-453.

Haane, G., 05.—Ueber die Cardiadrüsen und die Cardiadrüsenzone des Magens der Haussäugetiere. Arch. f. Anat. und Entwcklngsgesch., Leipz., Heft. I, 1-78.

Hamburger, E., 89.—Beitrage zur Kenntnis der Zellen in den Magendrüsen. Arch. f. mikr. Anat., Bonn, XXXIV, 225-235.

Hammerschlag, A., 96.—Untersuchungen über das Magencarcinom. Arch. f. Vrdngskrankh., Berl., II, 1, 198 et seq.

Hári, P., 01.—Ueber das normale Oberflächenepithel des Magens und über Vorkommen von Randsaumepithelien und Becherzellen in der menschlichen Magenschleimhaut. Arch. f. mikr. Anat., Bonn, LVIII, 685-726.

——— 92.—Revue de l'anatomie pathologique de la gastrite chronique. Gaz. hebdr. de med., Par., 33-34.

Hayem, G., 94.—Gastritis Parenchymatosa. Allg. Wien. Med. Ztg., 2-17.

Heidenhain, R., 70.—Untersuchungen über den Bau der Labdrüsen. Arch. f. mikr. Anat., Bonn, VI, 368-406.

Hemmeter, J. C., 98.—Ueber die Histologie der Magendrüsen. Arch. f. Vrdngskrnkh., Berlin, IV, 23.

Hertwig, O., 98.—Die Zelle und die Gewebe. 2 Buch. Kap. XIV, Jena.

Holmgren, E., 02.—Weiteres über die "Trophospongien" der Leberzellen und der Darmepithelzellen. Anat. Anz. Jena, XXII; 313.

Kohn, Alf., 02.—Das Chromaffine Gewebe. Ergbnsse. d. Anat. und Entwcklgsgech., Merkel u. Bonnet, Wies, XII, 253 et seq.

Kolossow, A., 98.—Eine Untersuchungsmethode des Epithelgewebes besonders der Drüsenepithelien und die erhaltenen Resultate. Arch. f. mikr. Anat., Bonn, LXII, 1-13.

Korcynsky, E. und Jaworsky, W., 02.—Ueber einige bisher wenig berücksichtigte klinische und anatomische Erscheinungen im Verlauf des runden Magenschwüres und des sog. sauren Magenkatarrhs. Deutsche Arch. f. klin. Med. Berl., XLVII, Heft. 5 und 6, 578.

Krukenberg, R., 88.—Ueber diagnostische Bedeutung des Salzsäurenachweiss bei Magenkrebs. Dissertation, Heidelberg, J. Horning, 1-73.

Von Kupffer, C., 83.—Epithel und Drüsen des menschlichen Magens. Festschr. d. ærztl. Ver., Munchen, etc., 27-46.

Langendorff, O., und Laserstein, S., 94.—Die feineren Absonderungswege der Magendrüsen. Arch. f. d. ges. Physiol., LV., 578-589.

Langley, J. N., 81.—On the Histology and Physiology of Pepsin-Forming Glands. Phil. Tr. Lond., CLXXII, part 3, 663-711.

Letulle, M., 00.—Anatomie pathologique générale des lesions inflammatoires. Pathologie général, Bouchard. III, 2 part, 553-642.

Leuk, 99.—Untersuchung zur pathologischen Anatomie des menschlichen Magens mit Berücksichtigung der praktischen Verwertbarkeit anatomisch diagnosticirter Magenschleimhaut Stückchen. Ztsch. f. klin. Med., Berl., XXXVII, 296 et seq.

Labarsch, O., 97.—Achylia Gastrica. Martius F. and Labarsch, O.

MacCallum, A. B., 05.—On the Distribution of Assimilated Iron Compounds other than Hæmoglobin and Hæmatins in Animal and Vegetable Cells. Quart. J. Mic. Sc., Lond., XXXVIII N. S., 175-274.

Mayer, P., 97.—Ueber Schleimfarbung. Mitt. a. d. zool. Station zu Neapel, Leipz., XII, 303-330.

Meyer, G., 89.—Zur Kenntniss der sogenanten "Magenatrophie." Ztsch. f. klin. Med., Berl., XVI, 366-392.

Müller, E., 92.—Zur Kenntniss der Labdrüsen der Magenschleimhaut. Biol. Fören. Förhandl., Verhandl. d. biol. Ver. in Stockholm, IV, No. 8.

Nussbaum, M.—Ueber den Bau und die Thätigkeit der Drüsen.

77.—1. Mitteilung. Die Fermentbildung in den Drüsen. Archiv. f. mikr. Anat., Bonn, XIII, 721-755.

78.—2. Mitteilung. Die Fermentbildung in den Drüsen. Archiv. f. mikr. Anat., Bonn, XV, 119-933.

79.—3. Mitteilung. Die Fermentbildung in den Drüsen. Archiv. f. mikr. Anat., Bonn, XVI, 532-544.

82.—4. Mitteilung. Archiv. f. mikr. Anat., Bonn, XXI, 296-351.

Pawlow, J. P., 02.—The Work of the Digestive Glands. Trans. into English by W. H. Thompson, London.

Pilliet, A., 87.—Sur l'évolution des cellules glandulaires de l'estomac chez l'homme et les vertébrés. J. de l'anat. et physiol., &c., Par., V, 463-497.

Pirone, R., 04.—Recherches sur la fonction sécrétoire des cellules glandulaires gastriques. Ztsch. f. allg. Physiol., Jena, IV, 62-78.

Popoff, P. M.—97.—Ueber Magenkatarrh. Ztsch. f. klin. Med., Berl., XXXII, 389.

Rabl, H., 91.—Die Entwickelung und Structur der Nebennieren bei den Vögeln. Arch. f. mikr. Anat. und Entwcklngsgsch., XXXVIII.

Rawitz, B., 99.—Bemerkungen über Karminsäure und Hämetein. Anat. Anz., Jena, XV, 437 et seq.

Sachs, A., 87.—Zur Kenntniss der Magenschleimhaut in krankhaften Zuständen. Arch. f. exper Path. u. Pharmakol., XXIV, 109 set seq.

Schaffer, J., 97.—Beiträge zur Histologie menschlicher Organe. Sitzungsb. d. k. Akad. d. Wissensch., Math-naturw., CI, CVI, 3 Abt., 353-455.

Schmaus, H. und Albrecht, E., 95.—Degenerationen, Ergbnsse d. allg. Path. u. patholog. Anat., Lubarsch und Ostertag. I, Heft 2. 151-166.

Schmidt, A., 95.—Ein Fall von Magenschleimhautatrophie nebst Bemerkungen über sog. schleimige Degeneration der Drüsenzellen des Magens. Deutsche Med. Wchnshcr. XXI, 300-304.

——— 95.—Untersuchungen über des menschliche Magenepithel unter normalen und pathologischen Verhältnissen. Arch. f. patholog. Anat., &c., Berl., CXLIII, 477-508.

Sewall, H., 79.—The Development and Renegeration of the Gastric Glandular Epithelium during fœtal Life and after Birth. J. Physiol. Lond., I, 321-334.

Solger, B., 96.—Ueber den feineren Bau der Glandula submaxillaris des Menschen mit besonderes Berücksichtigung der Drüsengranula. Festschr. z. 70. Geburtstag, Carl Gegenbaur, II, 179-248.

Stintzing, R., 99.—Zur Structur der Magenschleimhaut. Festschr. z. 70. Geburtstag von Carl von Kupffer, Jena, 53-56.

Stöhr, P., 82.—Zur Kenntniss des feineren Baues des menschlichen magenschleimhaut. Arch. f. mikr. Anat., Bonn, XX, 221-245.

Théohari, A., 99.—Etude sur la structure fine des cellules principales, de bordure, et pyloriques de l'estomac a l'état de repos et a l'état d'activité sécrétoire. Arch. d'Anat. micr., Par., III, 11-34.

Théohari, A. et Babes, A., 05.—Ueber ein gastrotoxisches Serum.: Centralbl. f. Bacteriol., u. Parasitenk., Jena, 1 Abt., XXXVIII, Heft 6, 663 et seq.

Tortora, C. J., 99.—Sulle cellule glandolari dello stomaco. Riforma med., II, 267 et seq.

Trinkler, N., 83.—Zur Kenntniss des feineren Baues der Magenschleimhaut insbesonders der Magendrüsen. Centralbl. f. d. med. Wissensch., X, 161-163.

Zimmermann, K. W., 98.—Beiträge zur Kenntniss einiger Drüsen und Epithelien. Arch. f. mikr. Anat., Bonn, LII, 546-551.

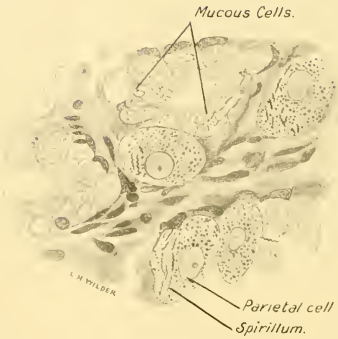


Fig. 1.

Fig. 1. Part of the bodies of glands near the line of union three months after gastroduodenostomy. The parietal cells are enlarged and many contain spirilla. Fixation: Kopsch's fluid. Stain: Neutral gentian by Bensley's method.

Fig. 2. Section of mucous membrane including the line of union four months after gastroduodenostomy. Fixation: Bouin's fluid. Stain: Mucicarmine. The gland cells which stained red with mucicarmine are shown black in the figure. (*See opposite page.*)


Duodenum
Stomach
Glands with only mucous and parietal cells in the bodies.
Glands with ferment cells in the bodies

Fig. 2.

FIG. 3. Section of mucous membrane, including part of the line of union six and a half months after gastroduodenostomy. Fixation: Kopsch's fluid. Stain: Neutral gentian by Bensley's method. Cells containing ferment granules are seen extending quite to the anastomosis. The glands containing them present the characteristics of normal glands of the fundus region. Ferment granules which stained blue in neutral gentian are shown black in the figure.

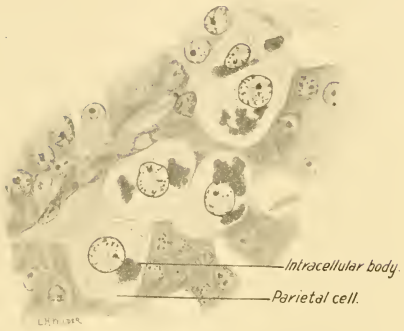


Fig. 4.

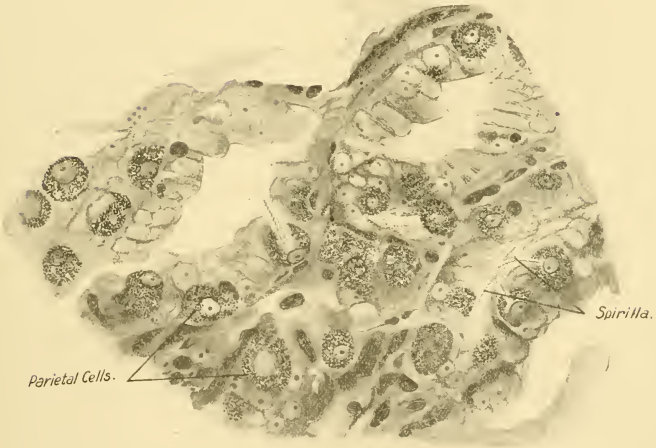


Fig. 5.

Fig. 4. Part of the bodies of glands near the line of union six and a half months after gastroduodenostomy. The parietal cells contain an irregularly distributed substance staining purple with neutral gentian. Fixation: Kopsch's fluid. Stain: Neutral gentian by Bensley's method.

Fig. 5. Part of the bodies of glands near the line of union ten months after gastroduodenostomy. Fixation: Kopsch's fluid. Stain: Copper-chrome-hæmatoxylin (Bensley).

EXPERIMENTS ON THE ORIGIN AND DIFFERENTIATION OF THE LENS IN AMBLYSTOMA.

BY

WILBUR L. LE CRON,

Student of Medicine.

Anatomical Laboratory, Johns Hopkins University.

WITH 5 PLATES.

Spemann[1] destroyed the rudiment of the optic vesicle on the wide open medullary plate of Rana fusca with a hot needle. When the eye failed to regenerate the lens was wanting, although the normal lens forming ectoderm had not been injured.

Lewis[2] cut away the optic vesicle in Rana palustris at a later stage, shortly after the closure of the neural folds, but before there were any signs of lens formation. The normal lens forming ectoderm was uninjured, but it failed to give origin to the lens, unless there was sufficient regeneration of the eye to bring it into contact with the ectoderm.

Lewis also transplanted the optic vesicles so cut away, beneath the ectoderm in other regions of the head. In embryos where such transplanted eyes came into contact with the overlying ectoderm, lens formation often occurred. His method of operation consisted in making an incision caudal to the eye region, turning the skin flap forward and cutting away the exposed optic vesicle, and then replacing the skin flap into its original position.

These experiments indicate very clearly that the lens is dependent for its origin upon the contact influence of the optic vesicle upon the ectoderm; the lens, in other words, is not a self-originating structure.

The beginning then of lens formation, namely, the thickening of the inner layer of the ectoderm to form the lens-plate, is dependent upon some influence exerted on this ectoderm by the optic vesicle. Is the continued differentiation of this lens-plate into the lens-bud, the lens-vesicle, and lastly the lens independent of any farther influence of the optic vesicle, or is the normal differentiation of the lens-plate dependent upon the continued influence of the optic vesicle and optic cup?

At the suggestion of Dr. Lewis, I undertook in the spring of 1905 an

[1] Ueber Correlationen in der Entwickelung des Auges. Verhandl. der Anat. Gesellschaft, 1901.

[2] Experimental Studies on the Development of the Eye in Amphibia. I. On the Origin of the Lens. Rana palustris. Am. Jour. of Anat., III, 1904.

experimental study of the question of the self-differentiation of the lens in Amblystoma punctatum. It seems possible that by removing the optic vesicle or optic cup at various stages before and during lens formation, one would be able to determine whether the continued influence of the optic vesicle was necessary for the normal differentiation of the lens.

The operation of cutting out or removing the optic vesicle without injury to the lens forming ectoderm or to the developing lens rudiment, is a simple one. The embryos were operated upon in tap water under the binocular microscope. They were held in position with a small pair of fine forceps, and a semi-circular incision was made, with a very finely pointed needle, through the ectoderm a little caudal to the bulge made on the side of the head by the developing eye. The skin flap was then turned forward, exposing the rounded optic vesicle. The latter was cut off from the side of the brain, and then when the skin flap was turned farther forward the optic vesicle was carefully pulled away from the ectoderm, sometimes as a whole, or else in small pieces. Great care was taken, however, not to injure the developing lens or surrounding ectoderm. After removal of the optic vesicle or the optic cup, the skin flap, which in later stages had attached to it the lens rudiment, was turned back into its original position, and held there by turning the embryo over on its side, with the skin flap against the bottom of the dish. The mere weight of the body sufficed to hold the flap in place. Healing was rapid; one to two hours generally being sufficient for complete closure of the wound. The operations were all made on the right side, while the left remained intact for purposes of comparison. The older embryos were first anesthetized in acetone chloroform in order to keep them quiet during the operation.

The embryos thus operated upon were allowed to live from two hours to thirty days, then killed in Zenker's fluid, thoroughly washed, embedded in paraffin, and cut into serial sections 10 micromillimeters in thickness. They were stained in hæmatoxylin and Congo red.

It has already been noted that in Rana fusca as well as in Rana palustris a lens will not arise from the normal lens-forming region of the ectoderm, if the optic vesicle is removed about the time of, or shortly after, the closure of the neural folds. Likewise in Amblystoma punctatum the lens fails to arise when the optic vesicle is removed at an early stage.

The optic vesicles were removed, by the operation already described, from embryos of Amblystoma shortly after closure of the neural folds (see Fig. 1). At this age there is not the slightest visible trace of lens formation or of any changes in the ectoderm leading to lens formation.

The optic vesicle is in contact with the ectoderm, but is not adherent to it (see Fig. 2).

Five embryos (Experiments $VII_{73\ 76\ 28\ 35\ 32}$) of this stage (VII) thus operated upon and killed 2, 4, 9, 10 and 12 days later, show no regeneration of the right eyes and likewise no signs of lens formation. In two embryos killed seven and nine days after the operation there appear partially regenerated eyes, which, owing to their small size and lack of contact with the overlying ectoderm, have failed to stimulate lens formation. Other embryos (Experiments $VII_{34\ 31\ 7\ 8}$) operated upon and allowed to live five, seven, and two, eight days, show regenerated eyes with developing lenses. In these the regenerated eyes were of sufficient size to come into contact or to remain in contact with the ectoderm long enough to cause lens formation. These latter experiments thus indicate that the operation of turning back the skin flap does not interfere with lens development, provided that the flap, when returned to its original position, comes into contact finally with a regenerated optic vesicle.

On the left or normal side of the embryo referred to above, which was killed two days after the operation, there is still no trace of the lens-plate, but on the normal side of the one killed four days afterwards, the lens-bud is well advanced, and in the embryos allowed to live five days, the lens-buds are still attached to the inner layer of the ectoderm and are about 110 microns in diameter. Embryos killed eight and nine days after the operation, however, show that the normal lens has completely pinched off from the ectoderm, and that the lens-fibers are beginning to differentiate at the medial pole. At eight days the lens is about 140 μ in diameter. The normal lens in the embryo allowed to live 10 days is about 130 μ in diameter and has pinched off from the ectoderm, and lens-fibers are fairly well developed. The normal 12 day lens is about 150 μ in diameter and shows still further differentiation.

The contrast, then, between no lens at all on the right side, where the optic vesicle is small or wanting even 12 days after the operation, and the normal lens on the left side is very marked indeed, and leads to the conclusion that the lens in Amblystoma as in Rana is not a self-originating structure.

In one embryo (Experiment VII_{33}) which was allowed to live 30 days after the operation, the right eye is entirely wanting, but in the region in which the lens would have formed under normal conditions, there seems to be a lens-bud rudiment (see Fig. 3). Its small size and rudimentary condition is in marked contrast to the normal lens on the opposite side of the head (compare Fig. 9). This little lens-bud, if it is one, and this seems somewhat doubtful, has probably arisen because the embryo at the time of the operation had advanced, as regards the formation of the

lens, farther than the other embryos of this series. That this is possible without any indication on the surface is very probable. In the examination of a number of embryos of Rana palustris, it was noted that embryos, which so far as external features were concerned seemed of the same age, often differed considerably in the amount of differentiation of the lenses. If this is true for Rana palustris it probably holds also for Amblystoma. Consequently some variations must be expected in the results obtained from embryos which are alike so far as their external features are concerned. And so, I am inclined to believe that the rudimentary lens-bud in the above experiment indicates that the optic vesicle had already exerted some influence on the ectoderm leading to lens formation, but when this influence was removed those ectodermal cells possessed but very little power of self-differentiation, and hence the development of the lens soon came to a standstill. That this is true will become more evident when we consider the results obtained after removal of the optic vesicle at later stages.

The foregoing experiments indicate very clearly then, that the lens is not a self-originating structure, and that it is dependent for its origin upon the influence or stimulus of the optic vesicle. If the optic vesicle is necessary for the starting of the lens, it may also be necessary for its differentiation, and if so, the removal of the influence of the optic vesicle during the various stages of lens development, should be followd by retardation and abnormal growth of the lens.

The optic vesicle was next removed from embryos at a stage (VIII) slightly older than those first operated upon. At this stage the tail-bud is just beginning to show and in most of the embryos the lens-plate appears as a very slight thickening of the inner layer of the ectoderm, where the optic vesicle comes into contact with it (Figs. 4 and 5).

Three embryos (Experiments $VIII_{40\ 17\ 36}$) thus operated upon and killed two, five and twelve days afterwards, were without regenerated eyes on the right sides and without traces of lens formation. On the normal or left side of the embryo killed two days after the operation there is a marked thickening of the ectoderm forming the lens-plate. The five-day embryo shows on the left or normal side a well-formed lens separated from the ectoderm and with the beginning of the formation of lens fibers. This normal lens is about 130 μ in diameter. The embryo that was allowed to live 12 days has on the normal side a well differentiated lens, about 190 μ in diameter, with long lens-fibers. Although the external appearances of these embryos would have indicated that the lens-plate had begun to form, nevertheless such thickenings on the under side of the skin flap were not observed at the time of the op-

eration. It is evident that the impulse before removal of the optic vesicle was not sufficient to bring about any visible signs of self-differentiation.

In another embryo (Experiment $VIII_{81}$) operated upon at this stage and killed three days afterwards, there is found on the left side a normal lens about 130 μ in diameter which has separated from the ectoderm, and on the right side a lens-plate—like thickening. At the time of the operation a very slight thickening of the ectoderm in the right lens region was noted. In this experiment no regeneration of the eye took place. There has evidently, then, been great retardation or almost complete stoppage in the growth and differentiation of this lens-plate, owing, I believe, to the absence of the continued influence of the optic vesicle.

Another embryo (Experiment $VIII_{72}$) of this stage operated upon and killed four days later has on the left side a lens-bud about 100 μ in diameter. The latter is still attached to the ectoderm. The right eye is wanting, but there is a thickening of the ectoderm in the normal position. The differentiation and growth of the right lens-plate has been much retarded, or perhaps has come to a standstill, evidently again through the loss of the influence of the optic vesicle.

Another embryo (Experiment $VIII_{69}$), killed five days after only partial extirpation of the optic vesicle, shows on the left side the normal lens 130 μ in diameter, just about ready to separate from the ectoderm. The regenerated right eye is deeply seated and separated from the ectoderm by mesenchyme. The ectoderm over the eye is thickened into a lens-plate, but is much checked in development. Evidently the deeply situated, regenerated eye has exerted no influence upon the lens-plate. At the time of the operation a slight thickening of the ectoderm was to be seen.

Another embryo (Experiment $VIII_{75}$) of this stage (VIII), from which the right eye was entirely removed, was allowed to live six days. At the time of the operation there was to be seen on the right side a slight thickening of the ectoderm for the lens-plate. The normal lens on the left side, about 150 μ in thickness, has separated from the ectoderm and shows considerable differentiation of its lens-fibers (Fig. 6). In the normal position on the right side there is a vesicular body separate from, but close to, the ectoderm (Fig. 7). It consists of a single layer of high columnar epithelial cells surrounding a central cavity, and is about 110 μ in diameter. The appearance and general arrangement of the cells indicate very clearly that it is a lens-vesicle considerably retarded in development. It is also abnormal in that the medial pole shows only slight indication of the beginning of lens-fibers. Such elongation of the cells of the medial pole is always found, even before the normal lens separates from the ectoderm.

Practically the same conditions are to be found in an embryo (Experiment $VIII_{71}$) killed nine days after the operation. There is no sign of a right eye, the normal left lens is about 160 μ in diameter, and is farther advanced than the one above. On the right side in the normal position for the lens there is a spherical body about 100 μ in diameter. It consists of a single layer of high columnar cells surrounding a cavity. It is entirely separated from the ectoderm, but lies near to it. The medial pole shows no indication of the formation of lens-fibers beyond a slight elongation of the cells. At the time of the operation a thickening of the ectoderm was noted.

In another embryo (Experiment $VIII_{39}$), killed 30 days after the operation, there is complete absence of the right eye. Beneath the ectoderm of the lens region on this side is a very degenerated-looking lens, about 110 μ in thickness (Fig. 8). It consists of a layer of flat epithelial cells surrounding a spherical mass of irregularly arranged, degenerating lens-fibers. The mass is much vacuolated, but stains rather like the lens-fiber part of the normal lens. The latter is 260 μ in diameter (Fig. 9). This experiment affords a marked instance of incomplete development and differentiation on the part of the lens when the influence of the optic vesicle is removed.

From another embryo (Experiment $VIII_{37}$) of this stage, the optic vesicle was partially removed, and 10 days after the operation a well developed eye had regenerated. The right lens has normally grown and differentiated, being almost as fully developed as the lens on the left side. Such instances where regeneration has occurred show that the operation itself of turning the ectodermal flap forward, does not interfere with lens development, provided the flap, when returned to its original position, comes into contact with a regenerated optic vesicle. Hence, whatever retardation of the lens may occur, must be explained by reasons other than by the immediate results of the operation.

A series of 11 experiments was made upon embryos of a stage (IX) somewhat older than the above. A thickening of the inner layer of the ectoderm was perceptible, and the invagination for the developing lens could be seen from the surface (Figs. 10-11).

Two embryos (Experiments $IX_{77\ 78}$), killed eight days after the operation, show lenses on the left sides normally developed, being about 170 μ in diameter. On the right sides are small spheroidal bodies consisting of one or two layers of columnar epithelium surrounding central cavities. Figures 12 and 13 are from mesial sections of the abnormal and normal lenses of one of these eight-day embryos. The right abnormal lens shows that the lens-plate (as seen in Fig. 11) continued its devel-

opment for a short time after the operation to the formation of a lens-bud, and to its separation from the ectoderm as a lens-vesicle. Here its development was ultimately checked and no lens-fibers were formed. This lens-vesicle is much smaller than the normal lens, and its lack of differentiation is also very evident.

This abnormal lens-vesicle is about the same size as a normal lens which has just separated from the ectoderm (see Fig. 19). It does not, however, show nearly the amount of differentiation of such a lens, especially in the region of the median pole, which shows no formation of lens-fibers, as does the normal lens. The cells of the median pole of this abnormal lens are somewhat elongated, as compared with those of the lateral pole, but this difference already existed in the lens-plate at the time of the operation. It seems, then, that the development of the lens-plate has been more in the change of form than internal differentiation.

In two experiments ($IX_{45\ 41}$) in which the embryos were allowed to live 11 and 14 days, the lenses of the corresponding sides are approximately of the same size, and about the same amount of difference is found between the right and left lenses of each embryo. The lenses on the left measure about 190 μ (compare Fig. 39), while those on the right are much smaller, being only about 100 μ in diameter. The latter (Fig. 14) are small spheroidal vesicles, consisting of a single layer of high columnar cells, and lying close to the ectoderm. In the cavity of the vesicle there are a few detached ectodermal cells, showing signs of degeneration. The cells of the epithelial wall of the vesicle are healthy in appearance, and more like those of the anterior epithelial layer of a younger normal vesicle (as in Fig. 19), than the more flattened cells of the left normal lens of the same embryo. The cells of the median pole of the abnormal vesicle, as in the preceding experiment, are somewhat elongated, as compared with those of the lateral pole. This lens-vesicle, although six days older than the one shown in Fig. 12, is much smaller. This diminution in size may have been caused by injury to the lens-plate, although at the time of the operation no injury was noted. On the other hand the lens-plate may not have been quite so far advanced at the time of the operation as the lens in the preceding experiment, and hence fewer cells of the inner layer of the ectoderm were influenced to take part in the formation of the lens-vesicle. Or, again, it may be after a certain time these abnormal lens-vesicles decrease in size, perhaps by the migration of some of the cells into the vesicle. This point can only be settled by further experimentation.

When the optic vesicle is removed after lens formation has begun, the development of the latter is not checked directly after, or by the im-

mediate effects of the operation, but retardation results only later from the lack of influence of the optic cup. In other words, after removal of the optic vesicle, lens development still continues for a short time, the extent of the development depending upon the size of the lens-plate at the time of the operation. The lens seems to receive a sort of momentum from the contact influence of the optic vesicle, and this impulse or stimulus is sufficient to give the lens a limited amount of self-differentiation, even in the absence of the optic cup. In all cases where long enough time is allowed, the process of separation of the lens from the ectoderm takes place, although generally the lenses lie nearer to the ectoderm after their separation than the normal ones.

Three other embryos (Experiments $IX_{20\ 42\ 44}$) of this stage (IX), which were killed six, eight, and thirty days after the operation, show slight regeneration of the right eyes. In the six-day embryo, the right lens contains a mass of fibers surrounded by a complete layer of epithelium, and no medial pole is distinguishable. In the other two instances the regeneration of the eye was very slight, and the lenses are not only considerably retarded in growth, but also in differentiation. In the 30-day embryo the difference in size of the two lenses is 120 μ, the left measuring 260 μ and the right only 140 μ in diameter. The latter contains some fibers and material staining like the normal lens. This material seems to be degenerated lens-fibers. Owing to slight regeneration of a few optic vesicle cells, the lens was perhaps at first rather normally developed and differentiated, but these few cells were not sufficient to influence the lens completely, and hence after a short time degeneration occurred.

In the next stage (X) the gill mass is large but shows no division. The lens-bud is well marked when the skin flap is turned forward in the operation (Figs. 15-16).

In one embryo (Experiment X_{26}) that was allowed to live 14 days after the operation the normal lens measures 160 μ and the right one 100 μ in diameter, thus showing a difference of 60 μ in size. The right lens has separated from the ectoderm but the eye is entirely wanting. A complete layer of epithelium surrounds the lens, which contains besides degenerated material a few healthy fibers in the region that had once been the medial pole (Fig 17, and compare Fig. 27).

In three other experiments ($X_{23\ 22\ 24}$), in which the embryos were allowed to live seven, nine, and eleven days, slight regeneration of the right eyes has taken place. In the seven-day experiment the regeneration is very slight indeed. The right lens is fairly normal in appearance, but somewhat retarded (Figs. 18-19). It has separated from

the overlying ectoderm, and is of good size, but is not so far advanced in point of differentiation as the normal left lens. A well defined medial pole is to be seen, but this appears to have stopped development when compared with the rapid advancement of the pole of the opposite lens to form fibers. Had the embryo lived longer the lenses would have shown a much greater difference, due, as in other instances, to the absence of the influencing optic cup. In the nine- and eleven-day experiments the right lenses have separated from the ectoderm. They have epithelial coverings one layer in thickness, and in the centers are masses of lens-fibers more or less degenerated. The normal lenses on the left sides measure about 170 μ, while the right lenses are much smaller, being only 120 μ in diameter.

In such instances, where only a slight regeneration of the eye occurs, the lenses may at first be rather normal in appearance, but later they invariably show degeneration, which is due, I believe, to the removal of the optic cup. The partial influence of the bit of the optic vesicle is not sufficient to further lens development. The experiments in which only partial regeneration occurred are likewise in accordance with the conclusion drawn, that the optic vesicle is necessary for the subsequent differentiation of the developing lens.

In a still older stage (XII) the gill mass shows three divisions, and the tentacles have begun to develop. The lens-vesicle is quite prominent. It has pinched off from the inner layer of the ectoderm, but is still tightly pressed against the same (Figs. 20-21). Twelve experiments were made upon embryos of this age, and five of these show some regeneration of the eye.

An embryo (Experiment XII_{51}) of this series was killed two hours after the operation. Within this time the skin had healed at the place of incision. No special change had taken place in the right lens, and it appears perfectly normal. Both lenses are still in contact with the overlying ectoderm, and show well defined medial poles with the beginning of lens-fiber formation (Figs. 22-23).

In another embryo (Experiment XII_{46}) which was allowed to live two days, the lenses have just separated from the ectoderm, and show no special difference in development (Figs. 24-25), although from the appearance of the right lens the loss of influence of the optic cup is just beginning to be felt. Its medial pole is very definite, but the shape and arrangement of the nuclei are somewhat abnormal.

Instances like the above indicate further that the operation of turning the skin flap forward does not interfere with the developing lens, which always has a certain amount of independent self-differentiation even

after removal of the optic cup. Hence, whatever retardation of the lens rudiment may occur is not to be explained by the immediate results of the operation.

In an embryo (Experiment XII_{53}) killed six days after the operation, the right lens (Fig. 28) is somewhat smaller than the left one (Fig. 27). It is rather normally developed and contains some healthy lens-fibers which have arisen from the medial pole. The pole, as it now appears, is not as broad as normal, but is becoming obliterated by the overgrowth of the layer of columnar epithelium, which surrounds the central mass of lens-fibers. Had the embryo lived longer, no doubt the medial pole would have entirely disappeared, and thus the lens would have been completely surrounded by this layer of epithelium, as in the following experiments.

Great retardation is shown in four of the experiments ($XII_{54\ 55\ 56\ 57}$) of this series (XII), of which two of the embryos were killed 10, and the other two 12 days after the operation. The right lenses are entirely surrounded by a single layer of cuboidal epithelium, and the medial poles from which the lens-fibers within had been formed are completely obliterated. The right lenses are rather small, and are not widely separated from the ectoderm. The lens-fibers adjacent to the epithelial covering seem rather healthy, while the central mass appears degenerated, but takes a similar protoplasmic stain. These lenses, then, differ considerably in structure as well as in size from the normally developed ones (Fig. 26, and compare Fig. 9).

In an experiment (XII_{56}) where the embryo was allowed to live 30 days, a bit of the optic cup had remained, but its influence, if any, did not long continue, for the right lens developed until it was only 150 μ in diameter, and then came to a standstill, and the lens-fibers within degenerated. A thin epithelial covering surrounds this vacuolated mass of degenerated fibers which are in marked contrast to the healthy fibers of the left lens. The normal lens measures about 250 μ in diameter, and thus shows considerable difference in size from the right one. In the experiments which continued over long periods of time, especially for 30 days, one is impressed not only with the retarded development of the right lenses, but also with the marked degeneration of the more highly differentiated lens-fiber tissue.

In three other embryos (Experiments $XII_{47\ 48\ 49}$) of this same stage (XII), the right lenses somehow came into contact with the nasal pits on their respective sides. The lenses have developed rather normally. It is possible that this peculiar arrangement was brought about by the operation. The skin flap perhaps became somewhat twisted, thus throwing the medial pole of the lens into contact with the nasal pit, which

thus has the appearance of influencing lens-differentiation. However, these few experiments prove nothing, but suggest the questions, whether the nasal pit is capable of influencing lens development or not, and whether the lens-fiber pole may be formed at different parts of the lens circumference.

In the following stage (XIII) (Fig. 29), the lens vesicle is quite well developed (Fig. 30), with a definite medial pole and the beginning of the formation of lens-fibers. It is completely divided off from the ectoderm, but still in partial contact with the same. It is found that the normal lens, after complete division from the inner layer of the ectoderm, still adheres or sticks to the same, and only after a certain time really separates from the ectoderm,—the processes of dividing off and separating not being simultaneous. Such a well advanced lens does not show at once any particular changes in development when the optic cup is removed. The lens-vesicle has evidently received something of a momentum from the continued influence of the optic cup, and now, after removal of this influence, develops for a short time apparently independently.

In an embryo (Experiment $XIII_{64}$) of this stage, killed four days after the operation, no particular difference between the two lenses is noticeable (Figs. 31-32). The medial poles are well defined and the lens-fibers within have a healthy appearance. The right lens is still in contact with the overlying ectoderm, but otherwise appears like the normal left lens. Both measure about 140 μ in diameter, and are perfectly developed. This experiment shows that the lenses of embryos of such a late stage (XIII) have power of considerable self-development after the removal of the optic cup. It requires some time before a marked difference in growth and in differentiation can be observed, but the difference invariably occurs, showing that the lack of influence of the optic cup is ultimately felt.

In an embryo (Experiment $XIII_{68}$) killed seven days after the operation, the left lens measures 170 μ and the right one 140 μ in diameter. The latter is thus somewhat smaller. It is well separated from the ectoderm, and contains normal lens-fibers. The medial pole, however, is rather obliterated by the epithelial covering of the lens (Fig. 33), and is not nearly so definite as in the normal lens (compare Fig. 13).

In a still older embryo (Experiment $XIII_{65}$) which was killed nine days after the operation, a greater difference is to be seen. The left lens is about 200 μ in diameter, while the right one measures only 150 μ, and is considerably smaller than the former (Fig. 34, and compare Fig. 9). It lies rather close to the ectoderm, and contains some healthy

lens-fibers. The medial pole is quite obliterated by the overgrowth of the layer of cuboidal epithelium that surrounds the lens completely.

In the oldest stage (XIV) operated upon, the lens was in very slight contact with the overlying ectoderm (Figs. 35-36).

The lenses of an embryo (Experiment XIV_{58}) allowed to live five days show no difference in size, both measuring 150 μ, but the right one has a complete covering of epithelium around the nuclear mass in the center (Fig. 37), and is not widely separated from the ectoderm. A small bit of the eye that had been left in contact with the lens, appears to have had little or no influence upon it.

The right lenses of embryos (Experiments $XIV_{60\ 61}$) killed eight and ten days after the operation are smaller than the normal, one is 20, and the other 30 μ less in diameter. Both right lenses (Figs. 38-40) are surrounded with complete layers of columnar epithelium. However, in the eight-day embryo there still remains an indication of the medial pole, while in the older embryo the pole is quite obliterated. The lenses are not widely separated from the overlying ectoderm, are smaller and in marked contrast to the perfect ones (Fig. 39). The fibers within the eight-day lens are fairly normal, but are beginning to show some degeneration, while those in the 10-day lens show considerably more.

An embryo (Experiment XIV_{59}) of this stage (XIV) that was allowed to live 30 days affords a very striking instance of the lack of development of the lens when the influencing optic cup is removed. The normal lens measures about 220 μ in diameter (compare Fig. 9), and is only 20 μ larger than the right lens. However, the latter consists only of a vacuolated mass of degenerated lens-fibers surrounded by a thin layer of epithelium (Fig. 41). The lens has separated completely from the ectoderm, but is still adjacent to same. It has grown considerably, but in order to accomplish perfect differentiation, the influencing medium of the optic cup was wanting, and the result was the extensive degeneration of the lens fibers with the complete obliteration of the medial pole.

Whether this continued influence of the optic cup upon the developing lens is a specific one or not, can be determined perhaps only by further experimental work. It may be that the nasal-pit, otic vesicle, or brain can exert such an influence, as to cause the lens to develop and differentiate normally. Lens-plates, lens-buds, lens-vesicles, and well differentiated lenses with some of the surrounding ectoderm might be transplanted into the brain itself, and thus determine whether there also the lens can find the influences essential to its normal differentia-

tion. Is the medial pole, destined for the formation of the lens-fibers, predetermined for a definite part of the developing lens, or can the pole be formed at any part of the circumference by modifying the surrounding influences at an early stage? Many interesting questions suggest themselves, some of which can be determined by experiment, while for others only hypotheses at present seem possible.

What the nature of this continued contact influence exerted upon the lens may be, is purely hypothetical. Perhaps, substances chemically formed in the protoplasm of the optic cup cells may be the important factors, which in some way are able to change the chemical nature of the lens cells, and thus promote their development into a normally differentiated lens. In the early stages of lens formation, the optic vesicle is in close contact with the developing lens, and there may be some kind of a protoplasmic connection, a relation of the two tissues, such that there may occur an interchange of protoplasm, or, what is more likely, of substances chemically formed in the protoplasm. This influence of the adjacent optic vesicle or optic cup, whatever may be the manner of its production, being chemical in nature or otherwise, probably continues throughout lens development and differentiation, and perhaps is even exerted to a certain degree upon the lens of the adult eye. However, the conditions of the lens in the older embryos are such that the effects of removal of the optic cup are only felt after some days. It is evident, then, that the age of the embryo indicates somewhat the extent of the effect produced by the removal of the optic vesicle. The lens may continue to grow some, even after disturbing these normal relations with the optic cup, but its growth is inevitably checked and is especially abnormal with regard to differentiation, due, undoubtedly, to the loss of influence, whatever its nature may be, of the optic cup.

CONCLUSIONS.

(1) A lens will not arise from the normal lens-forming area of the ectoderm without the contact influence of the optic vesicle. *The lens is not self-originating.*

(2) A lens will not develop from the lens-plate, lens-bud, or lens-vesicle, when the optic cup is removed. *The lens is not self-differentiating,* but is dependent upon the continued influence of the optic cup for its normal development.

(3) The older the lens rudiment at the time of removal of the optic cup, the greater the amount of independent differentiation the lens rudiment possesses.

(4) The lens rudiment ultimately ceases to develop after removal of the optic cup, and finally degenerates.

PLATE I.

FIG 1. Outline of an early stage of amblystoma, just after closure of the neural folds. First operative stage (VII). × 6½ diameters.

FIG. 2. Section through eye region of an embryo of the same stage as above (VII). Optic vesicle in contact with the ectoderm, which shows no signs of lens formation. × 45 diameters.

FIG. 3. Experiment VII_{33}.[3] Section through small abortive lens-like structure in the ectoderm in normal position for the lens. The embryo was killed 30 days after the complete extirpation of the optic vesicle. × 180 diameters.

FIG. 4. Outline drawing of an embryo of the second operative stage (VIII), showing first appearance of tail-bud. × 6½ diameters.

FIG. 5. Section through eye region of an embryo of the same stage (VIII) as above. The ectoderm shows some changes, as the beginning of the thickening of its inner layer for lens formation. × 45 diameters.

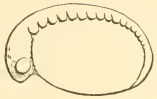
FIG. 6. Experiment $VIII_{75}$. Section through normal lens after 6 days. × 180 diameters.

FIG. 7. Experiment $VIII_{75}$. Section through right lens of above embryo 6 days after the complete extirpation of the optic vesicle, showing abnormal development as a resultant effect. At the time of the operation the lens-plate was visible as a mere thickening of the ectoderm. × 180 diameters.

FIG. 8. Experiment $VIII_{39}$. Section through the right lens of an embryo killed 30 days after removal of the optic vesicle. The lens shows marked retardation and lack of differentiation, and the lens-fibers are much degenerated. It measures about 110 μ in diameter, being much smaller than the normal lens. × 180 diameters.

FIG. 9. Experiment $VIII_{39}$. Section through normal left lens of above embryo after 30 days. 260 μ in diameter. (Contrast Fig. 8.) × 180 diameters.

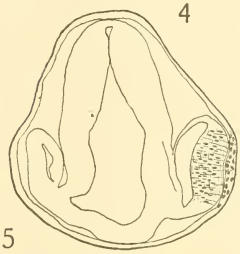
[3] The Roman numerals indicate the operative stage.



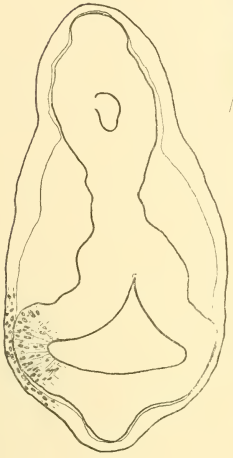
1

3

4



5

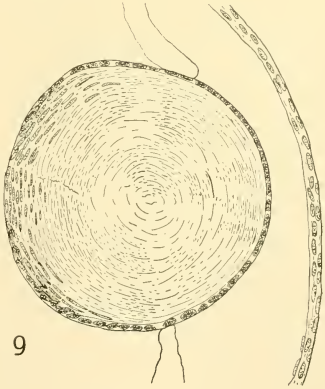


2

7

6

8



9

PLATE II.

FIG. 10. Outline drawing of an embryo of the third operative stage (IX). × 6½ diameters.

FIG. 11. Section through eye region of an embryo of the same stage (IX) as above, showing the lens-plate thickening of the inner layer of ectoderm. × 45 diameters.

FIG. 12. Experiment IX_{77}. Section through right abortive lens of an embryo 8 days after the removal of the optic vesicle. There is an indication of the medial-pole, but no definite lens-fibers are present. The lens measures 140 μ in diameter, being much smaller than the normal one below. × 180 diameters.

FIG. 13. Experiment IX_{77}. Section through left normal lens of above embryo after 8 days. Developed from lens-plate thickening of ectoderm (IX) to lens 170 μ in diameter. × 180 diameters.

FIG. 14. Experiment IX_{41}. Section through right abortive lens, showing degenerated ectodermal cells apparently, and no well defined medial-pole. The embryo was killed 14 days after the removal of the optic vesicle. × 180 diameters.

FIG. 15. Outline drawing of fourth operative stage (X), showing no division of the gill mass. × 6½ diameters.

FIG. 16. Section through eye region of an embryo of the above stage (X), showing a well-defined lens-bud. × 45 diameters.

FIG. 17. Experiment X_{26}. Section through right lens 14 days after the complete extirpation of the optic cup. The medial pole has disappeared, and only a few lens-fibers indicate its former position. The lens is small, measuring only about 100 μ, while the normal one is about 160 μ in diameter. × 180 diameters.

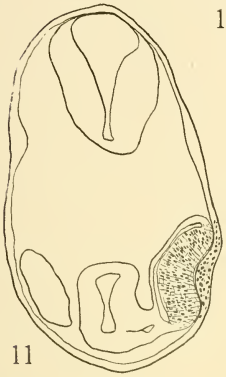
FIG. 18. Experiment X_{23}. Section through right lens of an embryo killed 7 days after the operation. The lack of influence of the optic cup has resulted in the retardation in growth and differentiation of the lens. × 180 diameters.

FIG. 19. Experiment X_{23}. Section through the left normal lens of the above embryo. × 180 diameters.

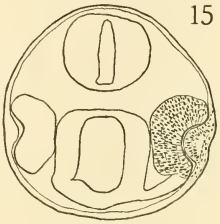
WILBUR L. LE CRON

10

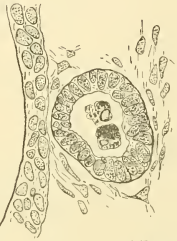
15



11

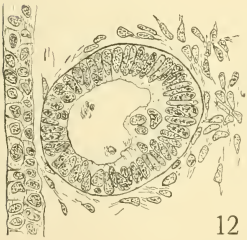


16



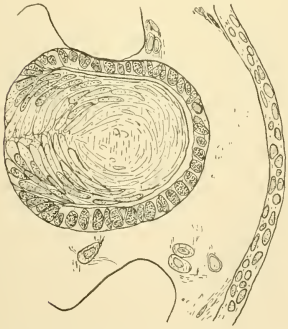
14

17

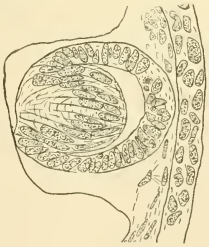


12

18



13



19

PLATE III.

FIG. 20. Outline drawing of the fifth operative stage (XII). The gill mass shows three divisions, and the tentacles are beginning to develop. × 6½ diameters.

FIG. 21. Section through eye region of an embryo of above stage (XII). The lens-vesicle is divided off from the ectoderm, but still in close contact with same. × 45 diameters.

FIG. 22. Experiment XII_{51}. Section through right lens-vesicle of an embryo killed 2 hours after the complete extirpation of the optic cup, showing no special changes within this time. × 180 diameters.

FIG. 23. Experiment XII_{51}. Section through left normal lens of above embryo, killed 2 hours after the operation. × 180 diameters.

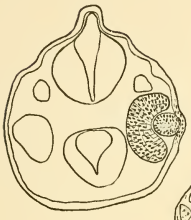
FIG. 24. Experiment XII_{46}. Section through the right lens-vesicle of an embryo killed 2 days after removal of the optic cup. The lens is similar to the normal one in size, 150 μ in diameter, and in differentiation. × 180 diameters.

FIG. 25. Experiment XII_{46}. Section through left normal lens of above 2 day embryo. × 180 diameters.

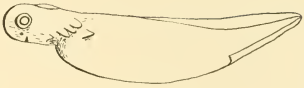
FIG. 26. Experiment XII_{50}. Section through right abortive lens 12 days after removal of the influencing optic cup. A complete layer of cuboidal epithelium surrounds the central mass of degenerating lens-fibers, and has obliterated the medial pole entirely. × 180 diameters.

FIG. 27. Experiment XII_{53}. Section through left normal lens of a 6-day embryo. × 180 diameters.

FIG. 28. Experiment XII_{53}. Section through right lens of the above embryo killed 6 days after the complete extirpation of the optic cup. It is separated from the ectoderm by mesenchyme, and the medial pole is almost obliterated by the overgrowth of the epithelial layer. × 180 diameters.

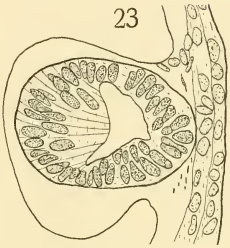


21



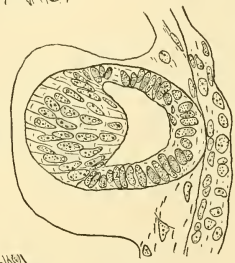
20

22

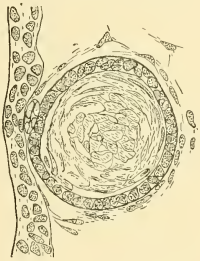


23

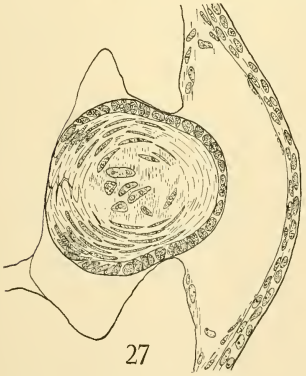
24



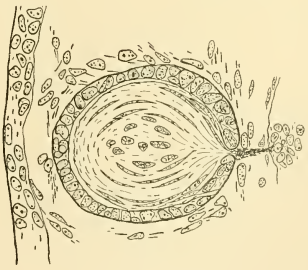
25



26



27



28

PLATE IV.

FIG. 29. Outline sketch of sixth operative stage (XIII). × 6½ diameters.

FIG. 30. Section through eye region of an embryo of same stage (XIII) as above. The lens-vesicle is well divided off from the ectoderm, but still adherent to it. × 45 diameters.

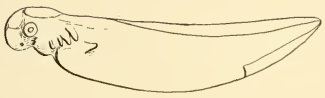
FIG. 31. Experiment $XIII_{64}$. Section through right lens of an embryo killed 4 days after removal of the optic cup. The lens appears perfectly normal, but is not widely separated from the ectoderm. × 180 diameters.

FIG. 32. Experiment $XIII_{64}$. Section through left normal lens of above 4-day embryo. The lenses both measure the same, being about 140 μ in diameter. × 180 diameters.

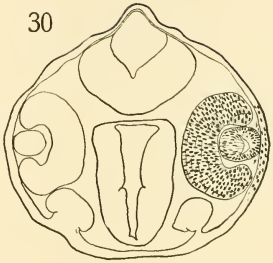
FIG. 33. Experiment $XIII_{68}$. Section through right lens, 7 days after the complete extirpation of the optic cup. It measures only about 140 μ in diameter, being about 30 μ smaller than the normal lens. The medial pole is obliterated, and the entire lens is surrounded by a single layer of columnar epithelium. × 180 diameters.

FIG. 34. Experiment $XIII_{65}$. Section through right lens of an embryo killed 9 days after the complete extirpation of the optic cup. The lens measures about 150 μ in diameter, and is about 50 μ smaller than the normal left lens. The medial pole is quite obliterated, and the mass of lens-fibers within are beginning to degenerate. × 180 diameters.

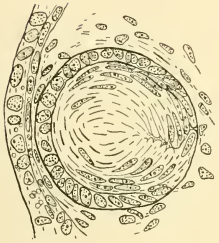
29



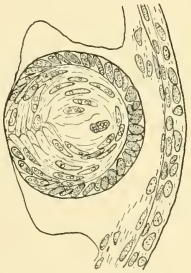
30



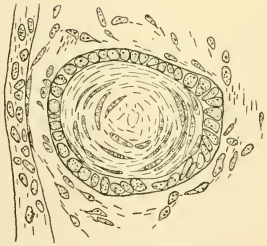
31



32



33



34

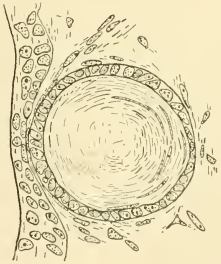


PLATE V.

FIG. 35. Outline sketch of seventh operative stage (XIV). × 6½ diameters.

FIG. 36. Section through eye region of an embryo of above stage (XIV). The lens-vesicle is but slightly adherent to the ectoderm. × 45 diameters.

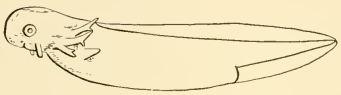
FIG. 37. Experiment XIV_{58}. Section through right lens, 5 days after the almost complete extirpation of the optic cup. The lens measures only about 150 μ in diameter. The lens-fibers appear rather healthy, but the medial pole has disappeared, owing to the complete layer of surrounding epithelium. × 180 diameters.

FIG. 38. Experiment XIV_{60}. Section through right lens of an embryo killed 8 days after removal of the optic cup. The lens is completely surrounded by a single layer of columnar epithelium, and measures about 160 μ in diameter. The lens-fibers are degenerating, but an indication of the medial pole still remains. (Contrast normal lens, Fig. 39). × 180 diameters.

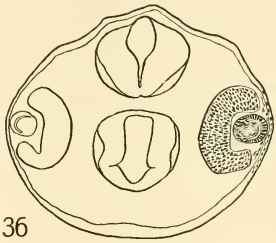
FIG. 39. Experiment XIV_{60}. Section through left normal lens of above 8-day embryo. The lens measures about 180 μ in diameter. × 180 diameters.

FIG. 40. Experiment XIV_{61}. Section through right lens, 10 days after removal of the influencing optic cup. It measures about 160 μ in diameter, being considerably smaller than the normal lens 190 μ in diameter, and shows no medial pole. × 180 diameters.

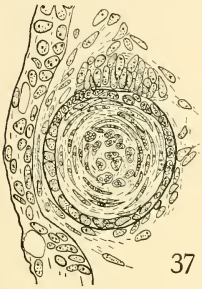
FIG. 41. Experiment XIV_{59}. Section through right lens of an embryo killed 30 days after the complete extirpation of the optic cup. The lens measures about 200 μ in diameter, while the normal lens of the opposite side is about 220 μ in diameter. It shows marked retardation in growth and differentiation, and is completely surrounded by a thin layer of epithelium, containing within a degenerating and vacuolated mass of lens-fiber material. (For contrast to left normal lens, see Fig. 9). × 180 diameters.



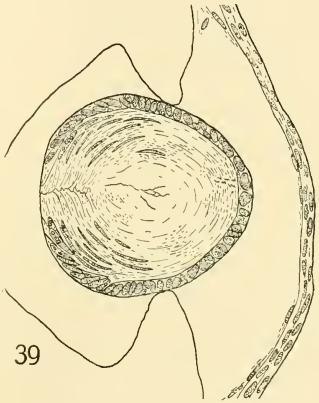
35



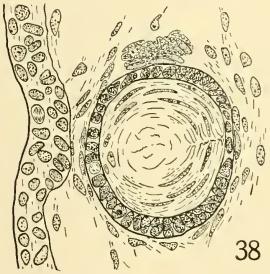
36



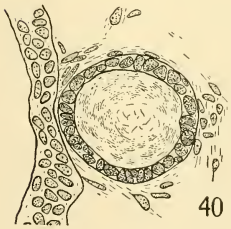
37



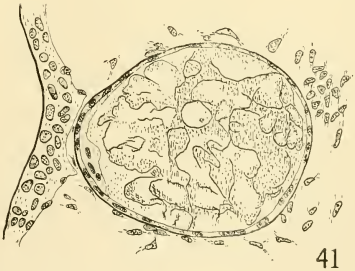
39



38



40



41

DEVELOPMENT AND VARIATION OF THE NERVES AND THE MUSCULATURE OF THE INFERIOR EXTREMITY AND OF THE NEIGHBORING REGIONS OF THE TRUNK IN MAN.

BY

CHARLES R. BARDEEN,

Professor of Anatomy, University of Wisconsin, Madison.

WITH 10 PLATES AND 7 TEXT FIGURES.

TABLE OF CONTENTS.

In a previous article in this journal (Bardeen and Lewis, 01), an outline was given of the early development of the limbs, body-wall and back in the human embryo. Lewis subsequently, 01, gave a more detailed account of the development of the arm, and I have recently, 05, described at some length the development of the spine and of the skeleton of the leg. The purpose of the following paper is a more detailed account of the development of the nerves and musculature of the leg and of the neighboring regions of the trunk and a consideration of the relation of developmental conditions to variations found in the adult. The embryological studies have been based chiefly on embryos belonging to the collection of Professor Mall of the Johns Hopkins University, who kindly placed them at my disposal. The statistical studies of nerve variation are based upon charts drawn from specimens in the dissecting rooms of the Johns Hopkins University and at the University of Wisconsin.

A. OUTLINE OF THE DEVELOPMENT OF THE MUSCLES AND NERVES OF THE INFERIOR EXTREMITY.

I. GENERAL FEATURES.

For a description of the development of the external form of the limbs and of the chief features which characterize the earlier stages in the internal differentiation, reference may be made to the three papers mentioned above. The posterior limb-bud is first seen as a massing of the mesenchyme at the posterior extremity of the Wolffian ridge, usually opposite the 21st to the 26th spinal segments. This mesenchyme arises in part from the axial mesenchyme, in part possibly from the somatopleure. There is no good evidence that in the mammals the myotomes contribute directly to it. On the contrary the myotomes are sharply marked off by a limiting membrane from the mesenchyme of the limb-

bud until this has become extensively developed. Afterwards this limiting membrane disappears, but there is little likelihood that cells derived from the myotomes then wander any considerable distance into the limb-bud. See Bardeen, 00. A capillary network connected with the umbilical artery and the cardinal vein is formed in the limb-bud at an early period. Somewhat later nerves extend into the limb. At the same time the mesenchyme begins to be differentiated into skeletal, muscular and dermal regions. During the development of the limb it shifts distally so that the distal margin of the limb-bud is brought opposite the 27th and 28th, and sometimes also the 29th, spinal segments. As this occurs, bundles of nerve fibres from these more distal spinal segments extend into the limb-bud to contribute to the posterior nerves of the limb. In the adult the most distal nerve to contribute to the nerves of the limb varies from the 26th to the 29th, but is most commonly the 28th. (Bardeen and Elting, 01). The number of spinal nerves contributing to the chief nerves of the limb varies from six to nine, but is usually seven or eight (Op. cit.). These variations are in all probability associated with variation in position of the limb-bud to the spinal axis during embryonic development.

The development of the main nerve trunks of the limb may be called the primary stage of nerve development and the associated variation in origin of the nerves, primary variation. As opposed to this primary development and primary variation we may call the growth which distributes the nerves within the limb the secondary stage of development and the variation there found secondary variation. During the primary period the spinal nerves send fibre bundles by direct paths to certain cutaneous areas and muscular anlages. During the secondary period the cutaneous nerves extend over the surface of the limb from the areas to which they are first distributed and the muscle anlages become differentiated into specific muscles to each of which nerve branches are given.

II. PRIMARY PERIOD OF NERVE DEVELOPMENT.

The general structural relations at the period when the nerves begin to extend into the limb-bud are shown in Plate I, Figs. 1 and 2. In Fig. 1 are shown the right limb and the distal half of the trunk from the 17th (9th thoracic) to the 29th (4th sacral) spinal segments in Embryo II (length 7 mm., age 26 days). The limb-bud lies opposite the 21st to the 26th spinal segments. The cœlom extends to a point opposite the 26th segment, but in the region of the limb it does not extend so far dorsally as in the thoracic region. In the figure several of the myotomes

of the left side, the axial mesenchyme, the aorta, the left cardinal vein, the intestines and the uro-genital organs are not shown. A portion of the right cardinal vein and a portion of the right umbilical artery are represented, reduced in size for the sake of clearness. The umbilical artery curves about the distal extremity of the cœlom. From the umbilical artery a branch passes into the limb-bud. Veins pass from the limb-bud into the cardinal vein. The blood-vessels of the limb exist at this time in the form of an irregular plexus.

The second, third and fourth lumbar nerves may be seen sending spreading bundles of nerve fibres into the dense tissue of the limb, dorsal to the cardinal vein. They extend, however, for no considerable distance into the limb-bud. The myotomes end abruptly near the base of the limb-bud.

Plate II, Fig. 1, represents the tissue differentiation in a section through the posterior limb-buds of Embryo II. At the left the bud is shown cut through an area near the distal extremity of the cœlom. At the right the cut is more dorsal and extends through the tips of the lumbar spinal nerves.

In Plate I, Fig. 2, are shown the right limb and the posterior half of the trunk from the 26th (8th thoracic) to the 30th (5th sacral) spinal segments in a slightly older embryo (CLXIII, length 9 mm.). Bundles of nerve fibres from the five lumbar and first two sacral nerves have become anastomosed into a plexus from which in turn four nerves have sprung. These represent the femoral, obturator, tibial and peroneal nerves. Within the limb the central mesenchyme, near the axis of the embryo, has become condensed. This condensed mesenchyme represents the femur and hip bone of the adult limb. In the drawing the outline of this sclerogenous tissue is made diagrammatically sharp. The femoral portion of the skeletal mass fades gradually into the undifferentiated mesenchyme of the distal portion of the limb. It is this skeletal mass which seems to divide the bundles of nerve fibres into the four main divisions which constitute the origin of the four chief nerves of the limb. The main artery and vein of the limb are represented at a reduced scale. The border vein at this period is well developed (see also Fig. C, Plate III of the article by Bardeen and Lewis, 01).

The differentiation of the tissue of the limb-bud, first noticed in a condensation of tissue in the region corresponding to where the femur projects against the hip girdle, is quickly followed by further changes. Externally there becomes visible a differentiation of the limb into foot-plate, crus and thigh, while within the limb-bud the further development

of the skeleton is marked by condensation of tissue, *scleroblastema,* to form the anlage of the skeleton of the foot, leg, thigh and hip girdle. About the scleroblastema is a myogenous zone, the *myoblastema,* composed of a slightly less dense tissue. In Embryo CIX, length 11 mm., this zone is best marked in the region of the hip (Plate II, Fig. 2). It is not clearly defined in the foot region. Between the myoblastema and the ectoderm lies a zone of less condensed tissue, the *dermoblastema.*

The chief nerves of the limb extend into the myoblastema. This is not a homogeneous layer. On the contrary from the time of its formation regions which represent the anlages of muscles or groups of muscles may be more or less clearly distinguished from regions which represent intermuscular spaces. In Plate III, Figs. 1 and 2, an attempt has been made to outline the muscle masses which represent the anlages of future muscle groups in Embryo CIX, length 11 mm. It is impossible to do this with exactness because the various regions are indefinitely bounded.

In this embryo the pelvic portion of the skeleton consists of a central region continuous with the head of the femur. From this central acetabular portion spring iliac, ischial and pubic processes. The femur is short and thick. The tibia and fibula are fairly definitely outlined, the foot-plate less definitely so.

The main nerve trunks have grown for a considerable distance into the limb. From them several of the chief muscular and cutaneous branches have sprung. The figures show these branches fairly well. In addition to the intrinsic nerves of the limb the anterior and posterior border nerves are also represented.

In Fig. 1 it may be seen that the myotomes in the region of the body wall have fused to form the anlage of the abdominal musculature. The lower margin of this extends distally about to the 21st spinal (1st lumbar) nerve. In Fig. E, Plate V of the article by Bardeen and Lewis, 01, it is represented slightly too short. From the ventro-posterior extremity of the abdominal musculature a somewhat indefinitely differentiated band of tissue may be followed to the pubic process of the pelvic girdle.

A slight communicating branch connects the twelfth thoracic with the first lumbar nerve. The main portion of this latter nerve extends forward on the internal surface of the distal margin of the anlage of the abdominal musculature and gives off a lateral, "iliac," branch. Ventrally the nerve divides into branches which represent the hypogastric and inguinal nerves. The 1st lumbar nerve also gives off a branch which passes to the lumbar plexus.

The obturator nerve arises from the first four lumbar nerves, passes through the obturator notch of the hip girdle and divides into two main divisions. Each of these terminates in a differentiated mass of tissue, the more anterior of which represents the adductor longus and brevis and the gracilis muscles, the more posterior, the obturator portion of the adductor magnus and possibly also the obturator externus muscle.

The tibial nerve arises from the fourth and fifth lumbar and first three sacral nerves. From it branches pass to muscle masses representing the obturator internus, quadratus femoris, hamstring, and the superficial and the deep posterior crural musculature. Distal to the tibial nerve the posterior cutaneous nerve of the thigh and the pudendal and caudal nerves may be seen.

In Fig. E, Plate V of the article by Bardeen and Lewis, 01, the urachus was represented much foreshortened in order to reveal the muscle masses of the leg. In Fig. 1, Plate III, the urachus is outlined in its true position as seen directly from the side.

In Plate III, Fig. 2, the genital and lumbo-inguinal nerves are seen passing ventro-laterally from the junction of the 1st and 2d lumbar nerves. The femoral nerve is seen passing outwards over the region of the acetabulum. It is surrounded laterally by the iliopsoas muscle mass and terminates in the quadriceps femoris muscle mass. From it arise lateral and anterior cutaneous branches, a branch which passes to the sartorius muscle mass, and the saphenous nerve.

The peroneal nerve arises from the 4th and 5th lumbar and first two sacral nerves, gives off branches for the anlages of the superior gluteal, inferior gluteal, short head of the biceps and peroneal muscle masses and terminates in the anterior crural muscle mass.

An idea of the relations of the main nerves as they enter the limb in Embryo CIX may likewise be gained from Plate III, Fig. 3. The pelvis, the abdominal and dorsal musculature, the lining of the body cavity, the border nerves and the main nerve trunks of the limb are here represented as viewed from in front. The femur and the main nerve trunks are shown cut in a plane somewhat distal to the head of the femur. The division of the main nerve trunks into separate branches for individual muscles is schematic.

III. MUSCLE DIFFERENTIATION.

At the period under consideration several possibilities of muscle differentiation must be considered. *1st.*—The tissue which represents the muscle masses just mentioned may extend into the limb-bud with the

nerves and become differentiated as the muscle branches are given off. The fact that Harrison, 04, has shown that in the tadpole muscle differentiation may take place when no nerves are developed makes this possibility highly improbable. *2d.*—The ingrowth of the nerves and the development of muscle branches may cause a "precipitation" of premuscle tissue about these branches. This likewise is rendered improbable by Harrison's experiments. *3d.*—Muscle differentiation begins in specific regions. Under normal conditions this differentiation begins simultaneously with the ingrowth of the nerves into the limb. Muscle branches extend into the differentiating musculature, owing perhaps to some specific attraction exerted upon the growing nerves. This seems on the whole to be the most probable course of development. The considerable variation shown in the origin and distribution of the nerves to the muscles renders it not improbable that their ingrowth is due in part to some special attraction exerted by the developing musculature upon the growing nerves, and variously responded to by the latter.

The paths opened up for the growth of the nerves to the muscles are, however, at first not as a rule in regions in which muscle tissue is to be differentiated, but in intermuscular areas. Thus the chief nerve trunks usually grow along paths which lie between main muscle groups. As the muscles of these various groups become differentiated the main nerve trunks of each muscle group are distributed in the septa which separate the individual muscles and finally after a nerve has entered the muscle for which it is destined it is usually distributed at first in the coarser intramuscular septa. During the early stages of development, however, the true muscle tissue cannot be sharply distinguished from the tissue which is to make up the skeletal framework of the muscle. For this reason it often appears as though the nerve to a muscle plunged at once into the midst of muscular tissue.

At a slightly later stage of development than that of Embryo CIX the differentiation of muscular tissue from the skeletal framework of the musculature is much better marked than in that embryo. Thus in Embryo CXLIV, length 14 mm., the individual muscles of the thigh may many of them be clearly distinguished (Plate II. Fig. 3). It may be seen in this embryo that although muscle differentiation in a given muscle is most clearly marked in the region where the respective nerve has come in contact with or has entered the muscle, the differentiation is not limited to this area but extends for a considerable distance toward the skeletal areas to which the muscle is to be attached. It is probable, however, that the differentiation of a given muscle begins as a rule in a

region which corresponds with the site of entrance of the chief nerve of that muscle. In Plate II, Fig. 3, several nerves and muscles are shown. The nerve to the gracilis muscle shows especially clearly. From this region the gracilis muscle may be traced in successive sections toward the pelvis and toward the tibia. The entrance of the inferior gluteal nerve into the gluteus maximus muscle also shows well in the figure. The two parts of the adductor magnus muscle, the obturator and sciatic portions, are shown near the site of entrance of the respective nerves. The semitendinosus muscle and the two heads of the biceps are shown cut at some distance from the site of entrance of nerves. About the two divisions of the sciatic nerve there is some dense tissue which probably does not, however, represent muscle tissue.

It is to be noted that during these earlier stages of muscle differentiation the muscle anlages are often connected at one extremity, less frequently at both extremities, with the skeletal anlages to which the muscle is subsequently attached. The tendons of the muscles are developed in continuity with the anlages of the muscles. As a rule the differentiation of the longer tendons begins in the vicinity of the muscle bellies and gradually extends toward the skeletal attachments.

In a considerably older embryo, CXLV, length 33 mm. (Plate II, Fig. 4), differentiation of the muscles is much further advanced. Not only the muscles but also the fasciculi are separated by a large amount of connective tissue. This shows especially well in the gluteus maximus muscle. The main branches of the nerves of the muscle may be followed in the larger intramuscular septa, the smaller branches in the smaller intramuscular septa. I have elsewhere described the intramuscular growth of nerves in the mammals (Bardeen, 00 and 03). It is of interest to note that after muscle differentiation is well under way there is relatively a much greater amount of connective tissue in the musculature of the embryo than in that of the adult.

After the stage of development exhibited by Embryo CIX the conditions within the limb become so complex that they can be better followed by tracing through the development of specific groups of nerves and muscles than by attempting to picture all the details of each successive stage of differentiation of the whole limb. In order, however, that the relations of specific groups of nerves and muscles to the general structural condition of the limb may be followed we shall first briefly describe the relations of the peripheral nervous system to the skeleton at two important stages of development.

IV. OUTGROWTH OF THE NERVES.

In Embryo CXLIV (length 14 mm.) the main nerve trunks are well developed as far as the foot. The relations of the nerves to the spinal column, abdominal musculature, skeleton of the limb and the surface of the limb are represented in Plate IV, Figs. 1 and 2. The 12th thoracic nerve sends a communicating branch to the first lumbar and from this latter arise the hypogastric and inguinal branches.

From the first lumbar nerve a branch is also given off to the lumbar plexus. From the 1st and 2d lumbar nerves arise genital and lumbo-inguinal branches. The femoral and obturator nerves arise from the 1st, 2d, 3d, and 4th lumbar nerves and give off the branches shown in the figures. The sciatic nerve, which arises from the 4th and 5th lumbar and first three sacral nerves, is composed for the greater part of its course of separate peroneal and tibial nerves. The various muscular and cutaneous branches are labeled in the drawing.

In Embryo XXII, length 20 mm. (Plate V, Figs. 1 and 2), the various nerves mentioned are much more highly developed than in Embryo 144. This difference of development is especially to be noticed in the feet. The figures indicate sufficiently well the relations of the nerves to the skeletal apparatus, the skin and the abdominal musculature.

A noteworthy fact brought out by these figures is that the cutaneous nerves are distributed at first to the anterior, distal and posterior margins of the embryonic limb, while the dorsal and ventral regions of the limb are given up to the differentiation of musculature.

Having thus considered in brief outline the more general features in the development of the muscles and nerves of the posterior limb we shall take up in turn a more specific study, first, of the development of the cutaneous nerves and then of that of the muscles.

B. DEVELOPMENT AND VARIATION OF THE CUTANEOUS NERVES.

Grosser and Fröhlich, 02, have given a good account of the development of the cutaneous nerves of the trunk. I have been unable to find any specific account of the embryonic development of the cutaneous nerves of the limbs, although the work of Sherrington, Head, and others on the segmental distribution of these nerves makes it of interest to inquire whether or not embryonic conditions can help to explain the phenomena these authors have described. In the following section the embryonic development and the variations in distribution of specific groups of nerves are first described and then the more general facts disclosed by this study are briefly reviewed.

I. ANTERIOR BORDER NERVES.

a. Development.

When the nerves begin to enter the limb-bud this lies, as pointed out above, usually opposite the five lumbar and first sacral nerves (Plate I, Fig. 1). The posterior margin of the developing body-wall and the anterior margin of the limb-bud usually overlap opposite the 21st segment. The nerves arising from the 21st spinal (1st lumbar) nerve are therefore true border nerves, being in part distributed to the abdominal wall and in part to the limb. The 20th and 22d spinal nerves (12th thoracic and 2d lumbar) also usually contribute to a greater or less extent to both regions, the 20th contributing to the cutaneous supply of the leg, the 22d slightly to the extreme margin of the abdominal musculature.

In Embryo CIX, length 11 mm. (Plate III, Figs. 1, 2 and 3), the border nerves are beginning to extend toward the skin. At this stage the oblique and the rectus muscles of the abdomen are beginning to be differentiated. The transversus muscle has not yet appeared. Between the ventro-anterior margin of the pubis and the ventro-caudal angle of the differentiating abdominal musculature a slight thickening of the mesenchyme represents the beginning of the tendon of the rectus and of the inguinal ligament. A considerable interval exists between the distal margin of the abdominal musculature and the anlage of the iliac crest. The musculature lies near the peritoneal cavity, while the crest is in the mesenchyme lateral to this cavity. Between body cavity and crest lies the femoral nerve with its branches (Fig. 3). From the first and second lumbar nerves the iliohypogastric and inguinal and the genital branch[1] of the genito-femoral extend ventrally between the cœlomic wall and the distal margin of the developing abdominal musculature. From the common trunk of the iliohypogastric and inguinal nerves a lateral branch, the "iliac," extends toward the skin in an area considerably anterior to the ilium. The lumbo-inguinal nerve and the lateral and anterior cutaneous branches of the femoral extend toward the anterior margin of the limb-bud.

In a slightly older embryo, CXLIV, length 14 mm. (Plate VI, Fig. 1) differentiation of the abdominal musculature has proceeded much further. The external oblique muscle is a thin sheet, somewhat wrinkled in the specimen. In the figure merely its origin from the lower ribs is shown. It extends distally into a sheet of mesenchyme which is thick-

[1] The term "genital" nerve is here used in preference to "spermaticus externus."

ened at its distal border into an embryonic inguinal ligament (lig. ing.). This latter extends from an anterior mesenchymatous process of the ilium toward the pubis. Ventrally it becomes continuous with the blastema of the pubic crest. Beneath the external oblique lies the internal oblique muscle. Distally this is connected by a mesenchymatous membrane with the inguinal ligament. In the figure merely the costal and inguinal portions of the muscle are shown.

The transversus muscle is differentiated immediately beneath the peritoneal membrane. It is not clear whether the material of the transversus musculature is derived from the cœlomic lining or from the myotomes. If from the latter the tissue wanders along the peritoneum from the region of the ribs.

At this early stage the anlage of the processus vaginalis may be seen in the form of a thickened mass of tissue which is continued from the plica gubernatrix through the internal oblique muscle and the aponeurosis of the external oblique above the inguinal ligament to the junction of the thigh with the trunk. Here it spreads out into processes which extend on the one side toward the mid line of the body, on the other toward the femur.

Between the transversus musculature and the internal oblique run the main trunks of the thoracico-abdominal nerves. The ilio-hypogastric and inguinal nerves pierce the internal oblique muscle and the aponeurosis of the external oblique much as in the adult. The iliac branch of the ilio-hypogastric, however, pierces the oblique muscles in a region anterior to its relative adult position. This is also the case in Embryo XXII, length 20 mm., Plate V, Fig. 1. Beyond the region of the inguinal nerve the cœlomic wall, backed by a thickened membrane representing the transversalis fascia, curves medially while the oblique musculature takes a somewhat lateral direction toward the inguinal ligament. Between the two is a space in which lie the femoral nerve, its proximal branches and the anlage of the ilio-psoas muscle. The genital branch of the genito-femoral nerve follows along the cœlomic wall almost parallel with the hypogastric and inguinal nerves but converging toward the latter. The point "X" in the figure represents a region where later the peritoneal wall will be pushed laterally over the ilio-psoas muscle so as to cover this and be brought in contact with the iliac crest. The lumbo-inguinal nerve passes out beneath the inguinal ligament in the vicinity of the femoral artery. It probably represents a lateral branch of the genito-femoral considered as the ventral division of a typical spinal nerve.

Ventrally the genital nerve, usually after anastomosing with the inguinal, passes along the vaginal process through the aponeurosis of the external oblique and over the inguinal ligament to the thigh. It is interesting to note that this development considerably precedes the descent of the testicle.

In Plate VI, Fig. 2, the border nerves of Embryo XXII, length 20 mm., are pictured. It is somewhat difficult to trace with certainty the border nerves in this embryo, but the figure is believed to illustrate approximately the actual relations. While in Embryo CXLIV a considerable interval separates the anlage of the iliac crest from the distal margin of the abdominal musculature, in Embryo XXII the crest is much further developed and at the same time has been rotated toward the dorsal portion of the distal margin of the oblique abdominal musculature. This at the same time has extended distally and become attached to the iliac crest. Meanwhile the peritoneal wall has bulged laterally so that the fascial extension of the transversus muscle covers the ilio-psoas muscle in the region of the pelvis and the transversus muscle has formed its pelvic attachments. The main trunks of the border nerves have been brought by these changes into relations which closely resemble those characteristics of the adult. Adult conditions are reached by some further relative shifting of parts and by the growth of the nerves within the areas for which they are destined.

The segmental relations of the border nerves may be best understood by comparing the position of the pelvic girdle when the nerves first extend toward the skin with the condition brought about by the shifting of the girdle. See Plates III, IV, V and VI. In Embryo CIX, the stage in which the nerves first extend toward the skin, the border nerves arise from the spinal nerves in the following order: iliohypogastric, inguinal, genital and lumbo-inguinal. As these nerves grow forward there takes place a rotation of the base of the limb medially, ventrally and posteriorly. At the same time the spinal column becomes straightened and the limb-bud as a whole descends posteriorly. The pubis is carried from a point opposite the 21st (12th thoracic) segment to a point opposite the 26th, and at the same time the posterior margin of the ilium is usually brought to lie opposite the 26th and 27th vertebræ to which it becomes attached. The two pubes are carried forward ventrally until they are united by the symphysis pubis.

As the pubis rotates ventrally and posteriorly the inferior portion of the abdominal wall is extended in a corresponding direction. The ven-

tral margins of the distal portion of the rectus muscles are brought into approximation when the symphysis pubis is formed. By the rotation of the hip bone the crest of the ilium is brought up against the dorsal portion of the distal margin of the abdominal musculature. The ventral portion of this margin becomes converted into the inguinal ligament. The courses of the abdominal nerves and the hypogastric and inguinal nerves are determined by their positions in the abdominal musculature. The genital nerve takes a more direct course towards its region of termination, although it too is usually bound up for some of the distal part of its course with the distal margin of the abdominal wall.

The peripheral region to which the lumbo-inguinal nerve extends is carried in a ventral, medial and posterior direction by the rotation of the limb. The main trunk of the lateral cutaneous nerve is caught by the rotating hip bone usually in the vicinity of the future anterior superior iliac spine and is carried up against the inguinal ligament. Thus by this rotation and shifting marked changes in the relative positions of the more anterior nerves arising from the lumbar plexus are brought about.

b. Variation.

A study of variation in the distribution of the fibre bundles of the spinal nerves to the various peripheral areas of the limb reveals the fact that any two nerves shown in Plate III, Figs. 1, 2 and 3, may be combined into a single trunk when they arise ordinarily in succession, but not otherwise. Thus the 12th thoracic and the hypogastric, the hypogastric and the inguinal, the inguinal and the genital, the lumbo-inguinal and the lateral cutaneous, the lateral cutaneous and the femoral, may be bound together for a greater or less part of their courses from the plexus to the limb. On the other hand, two or more nerve trunks may serve to convey fibres commonly carried in a single nerve. Separate iliac branches, extra lumbo-inguinal and genital nerves belong to this category as do also those "middle cutaneous" nerves which arise directly from spinal nerves, and the accessory obturator nerve. The frequency of variation of this sort in the border nerves I have previously described in this journal, **02.** In the same paper I have treated of the frequency of variation in segmental origin of the various border nerves. This is most marked. Thus the hypogastric nerve arose in 2% of instances from the 19th and 20th spinal nerves; in 32% from the 20th; 34% from the 20th and 21st; and 32% from the 21st. The iliac arose in 2.1% of instances from the 19th and 20th spinal nerves; 27.4% from the 20th spinal nerve;

37.1% from the 20th and 21st spinal nerves; and 32.7% from the 21st spinal nerve. The inguinal nerve arose from the 20th spinal nerve in 3.5% of instances; from the 20th and 21st in 38.3%; from the 21st in 51.5%; and was absent in 6.6%. The genito-femoral nerves arose from the 21st spinal nerve in 19% of instances; from the 21st and 22d in 79% of instances; and from the (21st) 22d and 23d in 2% of instances. In 1.2% of instances no lumbo-inguinal (crural) branch was found. It is probable that the variation in origin of the border nerves is due in part to a variation in position of the base of the limb-bud with respect to the spinal column, the more anterior spinal nerves serving to supply the limb when the limb-bud has a more anterior position at the time of the outgrowth of the spinal nerves. There is, however, no perfect correspondence between variation in origin of individual border nerves and that of the border nerves as a group.

In the same paper I showed that out of 133 instances, in 27 (20.30%) the lumbo-inguinal (crural) nerve emerged from the pelvis into the thigh in a lateral (external) region; in 81 instances (60.9%) in the middle (anterior) region; and in 25 instances (18.8%) in a medial (internal) region. After the nerve has passed into the thigh it may have a slight, a moderate or an extensive distribution to the skin. While this distribution usually corresponds to the region of exit, lateral, middle or medial, this is not always the case. For instance, a nerve emerging laterally may send a branch over to supply the fascia on the medial side of the leg. The following table indicates the frequency and extent of distribution of the lumbo-inguinal nerve to the skin of the lateral, anterior and medial portions of the thigh. By "lateral" region is meant an area lying lateral to a line drawn from the anterior inferior spine of the ilium to the lateral edge of the patella; by "medial," an area lying medial to a line drawn from the medial margin of the hip joint to the medial edge of the patella; and by "anterior," the intervening area. By "slight distribution" it is meant that by gross dissection the branches of the nerve could be followed but a short distance below the inguinal ligament; by "extensive distribution" it is meant that the branches could be followed readily over half way down the thigh. By "moderate distribution" is meant a distribution lying between these extremes. It will be understood, of course, that no hard-and-fast lines can be drawn between the various types of distribution tabulated. The table is intended merely to give an idea of the approximate frequency of distribution of the lumbo-inguinal nerve to approximate areas.

TABLE I.

Table Showing the Region and Extent of Distribution of the Lumbo-inguinal Nerve.

Type of Distribution.	Extent of Distribution. Slight. No. of inst.	Moderate. No. of inst.	Extensive. No. of inst.		
Lateral	5	8	6	19	15.4%
Anterior	11	36	8	55	44.7%
Medial	6	15	10	31	25.2%
Lateral and Medial			18	18	14.6%
	22 (17.9%)	59 (48%)	42 (34.1%)	123	

From this table it will be seen that the type of distribution most commonly met with is that of a moderate anterior distribution (36 instances, 29.2%). This corresponds to the distribution commonly given as the "normal" in the text-books and shown on the left side of the widely borrowed Léveillé figure given on Plate LIV of the Hirschfeld-Léveillé Neurologie.[2] The other types of distribution are, however, met with two thirds of the time. A study of the association of the types of distribution above given with race, sex and side of body, with various types of lumbo-sacral plexus and with variations in the spinal column has brought to light no intimate relations. The following table illustrates the relations of origin to distribution of the lumbo-inguinal nerve.

TABLE II.

Spinal Nerves from which the Lumbo-inguinalis arises.	Frequency of Types of Distribution of the N. Lumbo-inguinalis. Lateral. Slight.	Lateral. Mod.	Lateral. Extens.	Anterior. Slight.	Anterior. Mod.	Anterior. Extens.	Medial. Slight.	Medial. Mod.	Medial. Extens.	Lateral and Medial.
XX, XXI	..	2	..	..	5	1	..	..	3	..
XXI	1	1	1	..	4	3	..	1	2	1
(XX), XXI, XXII	..	1	1	1	5	..	2	3	..	3
XXI, XXII	3	3	2	8	18	3	4	10	5	3
XXII	1	1	..	2	3	..	..	1	..	..
(XXI), XXII, XXIII	1	..	1	..	..	2	..	..	..	1

From this it will be seen that there is slight relationship between the origin from the plexus and the distribution of this nerve. In case of

[2] Paris, 1853.

origin from the 23d spinal nerve the distribution is extensive in most of the instances studied.

The inguinal and genital nerves show relatively much less extensive variation in distribution. My data concerning the variation in their distribution as well as that of the iliac nerves are less accurate than those of the lumbo-inguinal so that the latter nerve may serve as an example of variation in the distribution of the border nerves.

II. CUTANEOUS NERVES OF THE FEMORAL GROUP.

a. Development.

By "cutaneous nerves of the femoral group" may be designated the lateral (external) cutaneous nerve of the thigh and the cutaneous nerves which usually spring directly from the femoral nerve. These nerves are all directed at first toward the anterior margin of the limb-bud. Figs. 1-3, Plate III, show their situation in an embryo of 11 mm. length. In Plate IV, Figs. 1 and 2, and Plate VI, Figs. 1, their position is shown in an embryo of 14 mm.

In this latter embryo (CXLIV) the lateral cutaneous nerve arises from the main trunk of the femoral, passes outwards through the anlage of the psoas muscle and approaches the skin near the junction of the anterior margin of the limb with the thigh. Several anterior and medial cutaneous nerves arise from the femoral nerve. The most proximal of these approaches the surface of the limb-bud somewhat more distally than the lateral cutaneous. A branch may likewise be followed through the anlage of the sartorius muscle and two through the septal tissue which divides the sartorius from the adductor group of muscles. The saphenous nerve passes between the anlages of the tendons of the sartorius and gracilis muscles to reach the subcutaneous tissue near the knee (Plate VI, Fig. 1).

In an older embryo, XXII, length 20 mm. (Plate V, Figs. 1 and 2, and Plate VI, Fig. 2), the further growth of the nerves just mentioned may be followed. The lateral cutaneous nerve has spread out in several branches toward the lateral surface of the thigh. The anterior and medial cutaneous branches have spread out over the antero-medial surface of the thigh, while the saphenous nerve has continued its growth toward the ankle. During the ventro-posterior rotation of the hip the lateral cutaneous nerve has been caught near the anterior superior spine of the ilium.

The further growth of these nerves to reach the conditions characteristic of the adult may easily be deduced by comparing Plate VI, Fig. 2.

with Plate VII, Fig. 1. The fascia lata which covers the lateral, anterior and medial cutaneous nerves for a considerable part of their course is just beginning to be differentiated in Embryo XXII.

b. Variation.

1. N. Cutaneus Femoris Lateralis.

The lateral cutaneous nerve in the adult usually springs by one or more roots from the lumbar plexus and takes a direct course through the psoas muscle and beneath the iliac fascia to a region near the anterior superior spine of the ilium whence it passes for some distance beneath the fascia lata and is finally distributed to the skin of the lateral region of the thigh.

The nerve varies considerably in origin. Out of 287 instances I found it arising in 39% from the 20th, 21st and 22d; in 43% from the 21st, 22d and 23d; and from the main trunk of the femoral in 18%. (Bardeen, 02).

The region where the nerve passes out into the thigh varies somewhat. It may be over the crest of the ilium just above the anterior superior spine or some distance below the latter. In two instances out of 146 it was found to emerge near the femoral nerve and then curve sharply outwards toward the lateral surface of the thigh. Rarely it is absent, its place being supplied by branches which spring directly from the femoral nerve below the inguinal ligament.

It varies considerably in extent of distribution. The distribution of the chief branches was found to be lateral to a line drawn from the anterior inferior iliac spine to the outer edge of the patella in 92 out of 146 instances (63%), Plate VII, Fig. 1. The area of distribution corresponds here essentially with that given as the normal one in most textbooks. In 45 instances (30.8%) the branches of the lateral cutaneous extended medially over the anterior portion of the thigh taking the place, to a greater or less extent of the anterior cutaneous branches of the femoral nerve. An instance of this sort is figured on the right side of the Léveillé figure mentioned above (p. 276). In 9 instances out of 148 (6.2%) a "lumbo-inguinal" branch, given off by the lateral cutaneous nerve, was distributed to the skin of the upper antero-medial region of the thigh. In two instances out of 148 the lateral cutaneous nerve was missing, its place being supplied by a large nerve which in origin, course through the psoas muscle and entrance into the fasciæ of the thigh resembled a lumbo-inguinal nerve. In two of the instances in which the "anterior or middle" distribution was extensive the lateral

cutaneous nerve passed into the thigh near the femoral nerve and then curved laterally towards the anterior superior spine. In one instance it gave a large communicating branch to the lumbo-inguinal nerve.

No relationship between race, sex or side of body and variation in the distribution of the lateral cutaneous nerve is apparent in the charts. An extensive distribution on the front of the thigh is somewhat more often associated with anterior than with posterior forms of lumbo-sacral plexus. This may be seen from the following table.

TABLE III.

Type of Plexus from which the N. Cut. Fem. Lat. arose:				Frequency of Types of Distribution of the N. Cutaneus Femoris Lateralis:		
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Lateral.	Lateral and Anterior.	Lateral and Medial.
Ant.	A	XXIV	XXVI	1		
	B	XXIV	XXVII	6	8	
	C	XXIV chiefly to sacral plexus	XXVIII	22	15	1
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII	37	16	6
	E	(XXIV) XXV	XXVIII	8	4	1
Post.	F	XXIV	XXIX	8	2	1
	G	(XXIV) XXV	XXIX	10		

The extensive anterior type of distribution is also most frequently associated with an "abnormal" type of vertebral column, especially a short one, as may be seen in the following table.

TABLE IV.

Vertebræ of Spinal Column.	Distribution of Lateral Cutaneous Nerve.		
	Lateral.	Lateral and Anterior.	Lateral and Medial.
7c 11t 5l 5s 4c		4	
7c 11t 5l 6s 2c	2	2	
7c 12t 4l 6s 3c		2	
7c 12t 4l 5s 3c		1	
7c 12t 5l 4s 2c		1	
Rudimentary 12th rib	19	9	2
Normal	29	11	7
7c 12t 5l 6s 3c	1	1	
7c 12t 6l 5s 3c	2	3	
7c 13t 5l 4s 3c	2	2	

The extensive anterior type of distribution is also more frequently associated with an anterior origin of the lateral cutaneous nerve than with a posterior origin. This is indicated in the following table.

TABLE V.

Spinal Nerves from which Lateral Cutaneous Nerve arose.	Distribution of Lateral Cutaneous Nerve. Lateral. No. of inst.	Lateral and Anterior. No. of inst.	Lateral and Medial. No. of inst.
(XX) XXI XXII	10	8	2
XXI XXII	18	10	1
XXII	9	3	
(XXI) XXII XXIII	25	13	5
XXII XXIII	5	3	1
XXII XXIII	10	3	
Trunk of femoral nerve	15	3	

The relations existing between the various types of distribution of the lateral cutaneous nerve and the various types of distribution of the lumbo-inguinal nerve are shown in the following table.

TABLE VI.

Types of Distribution of Lateral Cutaneous Nerve.	Types of Distribution of the Lumbo-inguinal Nerve. Lateral. Sl't.	Mod.	Ext.	Anterior. Sl't.	Mod.	Ext.	Medial. Sl't.	Mod.	Ext.	Lat. and Med.	Wnt'g.
Lateral	2	5	1	4	21	4	3	4	0	9	1
Lateral and anterior	1	2	2	4	3		1	7	7	4	2
Lateral and medial.	1	1		3	1		1		1		1
Wanting			2			1					

The most striking feature brought out by this table is the frequent association of an extensive anterior distribution of the lateral cutaneous nerve with a moderate or extensive medial distribution of the lumbo-inguinal nerve. This is shown in the Léveillé plate referred to above. This extensive distribution on the thigh of nerves derived directly from the 21st and 22d spinal nerves is, as has been pointed out above, most frequently associated with an anterior type of lumbo-sacral plexus and this in turn probably with a somewhat anterior position of the limb-bud at the time of the ingrowth of nerves. When the 21st and 22d spinal nerves are called upon to furnish a greater supply than usual of nerve fibres to the limb they are more apt to do so through direct paths (the lateral cutaneous and lumbo-inguinal nerve trunks) than through the more indirect route of the femoral nerve and its branches. This feature

is further brought out in the not infrequent association with the anterior forms of plexus of a direct anterior cutaneous branch from the plexus to the front of the thigh.

2. Separate Anterior Cutaneous Nerves.

Nerves of this sort spring usually from the XXI and XXII spinal nerves, but also sometimes from the XXIII and very rarely from the XXIV as well. Henle considers them as varieties of the lumbo-inguinal. In their course, however, they usually, at least, lie beneath or deep in

TABLE VII.

Type of Plexus from which the N. Cut. Fem. Lat. arises:			Origin of Separate Anterior Cutaneous Nerve.	
Type.	Furcal Nerve.	Most Distal Spinal Nerve to Limb.	From (XXI) XXII Spinal Nerves. No. of instances.	From XXII XXIII Spinal Nerves. No. of instances.
B	XXIV	XXVII	1	
C	XXIV chiefly to sacral plexus	XXVIII	10	1
D	XXIV chiefly to lumbar plexus	XXVIII		2
Type of vertebral axis	7c 11t 5l 6s 2c		1	
	12th rib rudimentary		8	1
	Not recorded		2	1
	Normal			1

the psoas muscle and beneath the iliac fascia instead of lying above the latter like the lumbo-inguinal nerve. A direct anterior (high middle) cutaneous nerve of the thigh was found 14 times in 123 instances (11.4%). It arose 11 times from the XXI and XXII spinal nerves and 3 times from the XXII and XXIII (once from a region opposite the XXIV spinal nerve). In the last instance entrance of fibres from the XXIV nerve was possible but was not certain. In all instances except two it arose in association with an anterior form of plexus. In all instances recorded except one the spinal axis showed a tendency to reduction by the presence of a rudimentary 12th rib and in one instance there were but eleven thoracic vertebræ. These facts are illustrated in the above table.

The following table illustrates the relation of a separate anterior cutaneous nerve of the thigh to the lateral cutaneous and lumbo-inguinal nerves.

TABLE VIII.

Types of Distribution of Lateral Cutaneous Nerve	Origin of Separate Anterior Cutaneous Nerve. From the XXI XXII Spinal Nerves. No. of instances.	From the XXII XXIII Spinal Nerves. No. of instances.
Lateral distribution	10	3
Wanting	1	
Types of Distribution of Lumbo-inguinal Nerve.		
Slight lateral	1	
Moderate lateral	1	
Extensive lateral	1	
Slight anterior	1	
Moderate anterior	2	2
Extensive anterior	1	
Slight medial	1	
Moderate medial	2	1
Lateral and medial	1	

From this table it will be seen that a separate anterior cutaneous nerve may be associated with a moderately developed lateral cutaneous nerve and with any form of distribution of the lumbo-inguinal nerve.

There is no indication that sex or race has influence on the frequency of development of this nerve. It was found more frequently on the left side than on the right, but this might not hold true were a greater number of instances studied. In only one instance was the nerve found on both sides of the same body. The following table indicates the race, sex and side of body in which the fourteen instances here studied were found.

TABLE IX.

Special Anterior Cutaneous Nerve from	White. Male. R	White. Male. L	White. Female. R	White. Female. L	Negro. Male. R	Negro. Male. L	Negro. Female. R	Negro. Female. L
XXI XXII sp. nerve	2	3		1	1	2		2
XXII XXIII sp. nerve		1				1	1	

The separate anterior cutaneous nerve is distributed on the thigh in company with branches derived directly from the femoral nerve. As a rule it is distributed in a territory separating that of the lateral cuta-

neous nerve from that of the branches of the femoral nerve, but occasionally it may have a more medial distribution. We may now pass to a consideration of the cutaneous branches of the femoral nerve arising beyond the inguinal ligament.

3. Anterior and Medial Cutaneous Branches of the Femoral.

The cutaneous branches of the femoral nerve to the thigh have been commonly divided by English and other anatomists into two groups, the "middle cutaneous" (nn. cutanei anteriores of Hénle) and the "internal cutaneous" (nn. cutanei medii of Henle). It is not possible always to draw a sharp distinction between these two groups of nerves.[3] In the most common form of distribution (Plate VII, Fig. 1) two "anterior cutaneous" nerves, a lateral and a medial, arise in the upper part of Scarpa's triangle. These branches descend in Scarpa's triangle, pass to the medial side of or through the substance of the sartorius muscle, pierce the fascia lata over the upper third of the sartorius muscle and are distributed to the skin of the lower two-thirds of the front of the thigh. The lateral branch pierces the sartorius muscle more frequently than does the medial branch. In place of two branches there may be three or only one. The "medial cutaneous" nerves arise as rami from one or more branches of the femoral nerve. The rami usually pass outwards in the septum between the sartorius muscle and the adductor group of muscles. Sometimes one or more of the rami pass through the substance of the sartorius muscle. The various rami supply the skin of the medial surface of the thigh and the more distal usually extend to the knee and join the saphenous and obturator nerves in supplying the medial side of the knee and upper part of the medial side of the back of the leg. There is great variation in the number and distribution of these rami of the "medial cutaneous" nerves. In two instances out of 80 a medial cutaneous nerve sent a branch as far as the ankle, parallel with the saphenous nerve.

Frequently the most proximal ramus of the medial cutaneous nerves on reaching the subcutaneous tissue, or even beneath the fascia lata turns back to take a course toward the region of distribution of the inguinal nerve (5 out of 80 instances).

The great variation in the number and territory of distribution of the anterior and medial cutaneous nerves of the thigh makes their statistical study both difficult and unsatisfactory. They vary in extent of distribution inversely with the lumbo-inguinal, lateral cutaneous, saphenous

[3] In the B. N. A. but one set of nerves is recognized, the *nn cutanei anteriores*. We shall here, however, adopt the Henle terms.

and obturator nerves, with branches of which anastomoses are usually formed.

Considering as a group the nerves which supply the front of the thigh it is found that the most common form of distribution is that of a moderately extensive lateral cutaneous nerve associated with two anterior and one or two medial cutaneous nerves the branches of which are distributed over the front and medial side of the thigh. As a rule the skin beyond the knee is supplied mainly by branches from the saphenous. This general mode of distribution was found in 64% of instances. In about 33% of instances there was an extensive distribution of the lateral cutaneous nerve with a more restricted distribution of the anterior and medial cutaneous nerves.

In the following table an attempt has been made to show the number of chief nerve branches distributed to the anterior surface of the thigh

TABLE X.

Cutaneous Nerves.	Number of Main Nerve Branches Distributed to the Anterior Surface of the Thigh.																														
N. cut. fem. lat...	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	4	4	4				
N. cut. fem. ant. from plexus.....	..	..	1	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
N. cut. fem. ant. from N. femoralis.....	2	1	1	..	1	..	1	2	2	2	2	2	3	3	1	1	2	2	2	2	3	1	1	1	2	1	1	2			
N. cut. med.....	1	1	1	1	1	2	2	3	2	1	2	1	1	2	0	1	1	2	1	2	1	1	1	2	1	1	2	1			
N. saphenous R. infrapatellaris..	1	1	1	1	1	1	0	2	0	1	1	0	1	1	1	1	1	1	2	0	1	0	1	1	1	1	1	1			
No. of instances..	1	1	2	6	1	2	1	1	3	5	3	1	1	1	1	6	14	2	3	1	2	1	2	3	13	3	2	5			

in 87 instances. The lateral cutaneous nerve has been counted as single when numerous small rami are given off from the main trunk; as double when the nerve divides into two main trunks before or soon after passing under the inguinal ligament; as triple and quadruple when it divides into three or four main nerve trunks. By separate anterior cutaneous is meant a branch arising directly from the plexus. The anterior cutaneous nerve is counted as single when one main trunk arises from the femoral nerve; as double when two separate trunks arise; and as triple when three such trunks arise. The same is true of the medial cutaneous nerve.

4. N. Saphenous.

The saphenous nerve is fairly constant in its general mode of distribution. The greatest variation comes in the distal extent of its distribution. In three instances out of 75 it was found to extend to the great

toe. Although in several instances students failed to trace the nerve further than the knee, I do not feel that their work is sufficiently accurate to give figures as to the frequency of extremely limited distribution of the saphenous nerve. I have seen no instances of the passing of the saphenous nerve to the back of the thigh through the adductor magnus muscle as described by Hyrtl (Henle, Nervenlehre, s. 573). As a rule the main trunk of the saphenous nerve passes skinwards between the tendons of the sartorius and gracilis muscles. The patellar branch of the saphenous usually passes through the substance of the sartorius muscle but may pass over the anterior margin of the tendon. Below the knee the saphenous nerve may be continued in one or two main trunks toward the ankle.

III. CUTANEOUS BRANCHES OF THE OBTURATOR NERVE.

The superficial branch of the obturator nerve may terminate in a cutaneous branch of variable size. In the embryos studied I have been unable satisfactorily to trace the development of this nerve. In the adult it usually passes distally between the gracilis and adductor longus and becomes superficial between the gracilis and sartorius muscles in the middle third of the thigh. It commonly anastomoses with branches either from the medial cutaneous nerves of the thigh or from the saphenous nerve or both, and helps to form the subsartorial plexus. The fibres of the cutaneous branch of the obturator may join the medial cutaneous or the saphenous nerve beneath the sartorius and be distributed in the branches of these nerves without giving rise to any independent branches. How constant the cutaneous branch of the obturator may be I have been unable satisfactorily to determine. Students dissecting frequently fail to find it. Owing to the fact that this may often be due to its small size the negative records cannot safely be used in making up statistics.

Out of 80 instances in which the nerves of the thigh were carefully charted, in 12 a large cutaneous branch passed from the obturator to the region of the knee and in 10 other instances one passed to or beyond the middle third of the crus. A well developed obturator branch to the skin is found more frequently associated with "normal" and "anterior" than with posterior types of lumbo-sacral plexus and relatively more frequently in white than in negro subjects.

IV. ACCESSORY OBTURATOR NERVE.

This nerve was not found in the embryos studied. Out of 250 plexuses in the adult it was found in 21 (8.4%). It seems to be especially frequently associated with the anteriorly situated types of plexuses. It was

found relatively more frequently in males (9.3%) than in females (5.4%) and in white (10.8%) than in negro subjects (6.4%). In most instances an anastomotic branch could be traced to the cutaneous branch arising from the obturator nerve. The following table shows the frequency with which accessory obturator nerves of various types of origin were associated with various types of lumbo-sacral plexuses.

TABLE XI.

Type of Plexus from which the N. Cut. Fem. Lat. arises.			Origin of Accessory Obturator.		
Type.	Furcal Nerve.	Most Distal Spinal Nerve to Limb.	From (XXI) (XXII) XXIII Sp. Nerves.	From (XXII) XXIII XXIV Sp. Nerves.	From XXIV Sp. Nerve.
B	XXIV.	XXVII.	1		1
C	XXIV chiefly to sacral plexus.	XXVIII.	5	2	1
D	XXIV chiefly to lumbar plexus.	XXVIII.	2	5	2
F	XXIV.	XXIX.	1		
G	(XXIV) XXV.	XXIX.	1		

V. CUTANEOUS NERVES OF THE SCIATIC GROUP

The cutaneous nerves originally extending toward the posterior and distal margins of the embryonic limb are greater in number and have a more extensive distribution than those of the anterior border. They consist of the posterior cutaneous nerve of the thigh, n. cutaneus femoris posterior (small sciatic), with its cluneal, perineal, hamstring and terminal branches, and the cutaneous rami which arise from the peroneal and tibial nerves. We may consider first the early embryonic development of these nerves and then the variations found in the adult.

a. Embryonic Development.

1. N. Cutaneus Femoris Posterior.

In Embryo CXLIV, length 14 mm., Plate IV, Figs. 1 and 2, two nerves may be seen extending out toward the posterior margin of the base of the limb. One of these nerves represents the posterior cutaneous nerve, the other either the perineal ramus (inferior pudendal) of that nerve or the perforating cutaneous nerve. The gluteus maximus muscle

does not at this period completely overlap the sciatic nerve and the two cutaneous nerves have a free path for growth. In Embryo XXII, length 20 mm. (Plate V, Figs. 1 and 2) but one cutaneous nerve can here be distinguished. This is clearly the posterior cutaneous nerve. In subsequent development the nerve is shifted from the posterior margin over a region corresponding to the original medial surface of the limb-bud and gives rise to extensive branches. In Embryo XXII perineal and cluneal rami may be traced for a short distance from the main trunk.

2. N. Suralis.

The main trunk of the sural nerve (external saphenous) may be seen arising, through the N. cut. sural medialis, from the tibial nerve in Embryo CXLIV (Plate IV, Figs. 1 and 2). In Embryo XXII, Plate V, Figs. 1 and 2, it is well developed and branches may be traced over the dorsum of the foot. The main trunk of this nerve at this period occupies a much more lateral position than subsequently. With the development and shifting of the gastrocnemius muscle the trunk of the nerve near its origin becomes shifted toward the middle of the calf and buried between the two heads of the gastrocnemius muscle.

3. N. Surae Lateralis.

In Embryo CXLIV (Plate IV, Fig. 2) this may apparently be recognized as short branch. In Embryo XXII (Plate V, Fig. 2) it is not much more highly developed. Subsequently it too becomes shifted over the back of the calf, but its branches are supplied to the original posterior margin of the limb (the external surface of the leg) as well as to the back of the leg. One of these branches finally anastomoses with the main trunk of the sural nerve. Variations on the adult indicate that the latter may at times arise from the N. suræ lateralis.

4. Nn. Peronei.

The superficial and deep branches of the peroneal nerve may readily be distinguished in Embryo CXLIV (Plate IV, Fig. 2) but the terminal cutaneous rami are not clearly developed. In Embryo XXII (Plate V, Fig. 2) these cutaneous rami may be followed over the dorsum of the limb-bud. They seem to have a simple direct growth toward the areas they are subsequently to supply.

5. N. Tibialis.

The terminal cutaneous branches of this nerve likewise cannot be distinguished in Embryo CXLIV (Plate IV, Fig. 1) but are clearly to be made out in Embryo XXII (Plate V, Fig. 1). Like those of the peroneal

nerve they seem to have a fairly direct path of growth. From the dorsal surface of the tibial nerve the calcaneal branch may be seen taking its rise.

b. Variation.

1. N. Cutaneus Femoris Posterior (small sciatic).

This nerve shows considerable variation in origin and distribution.

a. Origin from sacral plexus.—As stated in most text-books, it commonly arises from the 26th, 27th, and 28th spinal nerves (1st, 2d, and 3d sacral). It may, however, arise from the (25th) and 26th; (25th), 26th, and 27th; 26th and 27th; (25th), 26th, 27th, and 28th; 27th and 28th, or from the (27th), 28th, and 29th spinal nerves. In table XII the frequency of these various modes of origin is shown. It is possible that in some of the instances tabulated the origin of the posterior cutaneous nerve was more extensive than the tabulation charts show, because in tracing back a nerve to the spinal roots from which it springs, small bundles of nerve fibres are sometimes torn. It is believed, however, that the chief roots of the nerve are indicated in the charts from which the table was made. It is to be noted that while an anterior position of the roots of the posterior cutaneous nerve of the thigh usually corresponds with an anterior position of the lumbo-sacral plexus, this correspondence is not perfect.

In these variations no special relations to race, sex, or side of body are apparent in the charts tabulated.

According to A. Soulie (Poirier and Charpy, 01), the branch from the 2d sacral nerve to the posterior cutaneous nerve is constant while branches from the 1st and 3d are less constant and occasionally one may find a branch from the 4th sacral.

There is great variation in the extent to which the roots and the trunk of the posterior cutaneous nerve are bound up with neighboring nerve trunks, such as the inferior gluteal, sciatic and pudic nerves. As a rule, however, the union between these trunks is so slight that they may be readily separated. It has not seemed, therefore, worth while to attempt a tabulation of relations of this sort. Not infrequently (in about 25% of instances) the perineal rami arise from a trunk which springs by special roots from the plexus. See table XIII.

b. Distribution.—*Gluteal branches* (*nn. clunium inferiores*). These most commonly arise from a single branch given off from the posterior cutaneous nerve while this lies beneath the gluteus maximus muscle. This branch may pass up over the lower margin of the muscle before dividing into terminal rami (31 out of 77 instances) or it may divide into two or more rami which pass out under and turn back over the muscle in several

TABLE XII.

Type of Plexus from which the N. Cut. Fem. Post. arises.				Frequency of Origin of N. Cut. Femoris Post. from :							
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. (XXV) XXVI	Nn. Sp. XXV XXVI XXVII	Nn. Sp. (XXV) XXVI XXVII	Nn. Sp. (XXV) XXVI XXVII XXVIII	Nn. Sp. XXVI XXVII XXVIII	Nn. Sp. (XXVI) XXVII XXVIII	Nn. Sp. (XXVII) XX III XXIX	Total Number.
Ant.	A	XXIV	XXVI	1							1
	B	XXIV	XXVII		3	11		5	4		23
	C	XXIV chiefly to sacral plexus	XXVIII			14	2	25	20		61
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII		3	22		31	37	5	98
Post.	E	(XXIV) XXV	XXVIII			4	1	5	6	2	18
	F	XXIV	XXIX					4	4	6	14
	G	(XXIV) XXV	XXIX					6	5	5	16
Total Number.....				1	6	51	3	76	76	18	231

areas. In 33 out of 77 instances two such rami were found (Plate VII, Fig. 2); in 9, three; in 2, four; and in 2, five.

Perineal branches.—As a rule the perineal rami arise from a single trunk which branches from the posterior cutaneous nerve (70 out of 94 instances) or arises separately from the plexus and runs a parallel inde-

TABLE XIII.

No. of subject.	Race, sex, and side of body.	Type of plexus. See Table XII.	Origin of N. Cut. Fem. Post.	Origin of N. Perinealis.	Vertebral Column.
218	B, M, R	B	XXV, XXVI, XXVII	XXVI, XXVII	11t, 5l, 5s, 4c
269	W, M, L	B	XXVI, XXVII	XXVII	12th rib, short
395	B, F, R	B	XXVI, XXVII	XXVII	12t, 5l, 4s, 4co
607	W, M, L	B	?	?	12th rib, short
649	B, F, R	B	XXVI, XXII	XXVI, XXVII	12t, 4l, 5s, 3co
243	W, M, L	C	XXVI, XXVII, XXVIII	XXVII, XXVIII	12th rib, short
301	B, F, L	C	XXVI, XXVII, XXVIII	XXVII, XXVIII	12th rib, short
282	W, M, L	C	XXVI, XXVII, XXVIII	XXVII, XXVIII	12t, 5l, 6s, 2co
218	B, M, L	C	XXVII, XXVIII	XXVII, XXVIII	11t, 5l, 5s, 4co
476	B, M, R	C	XXVII, XXVIII	XXVII, XXVIII	12th rib, short
423	B, M, R	C	XXVI, XXVII	XXVII, XXVIII	11t, 5l, 6t, 2co
583	W, M, L	D	XXVI, XXVII, XXVIII	XXVII, XXVIII	Normal
405	W, M, R	D	XXVI, XXVII, XXVIII	XXVII, XXVIII	"
303	W, F, R	D	XXVI, XXVII, XXVIII	XXVII, XXVIII	"
211	B, M, L	D	XXVI, XXVII, XXVIII	XXVII, XXVIII	"
152	B, F, L	D	XXVII, XXVIII	XXVII, XXVIII	"
547	B, M, L	E	XXVI, XXVII, XXVIII	XXVII, XXVIII	"
108	B, F, L	E	XXVI, XXVII, XXVIII	XXVII, XXVIII	"
108	B, F, R	E	XXVII, XXVIII	XXVII, XXVIII	"
612	B, F, R	E	XXVII, XXVIII	XXVII, XXVIII	12t, 6l, 5s, 3co
282	W, M, R	F	XXVIII, XXIX	XXVIII, XXIX	12t, 5l, 6s, 2co
42	B, M, R	G	XXVI, XXVII, XXVIII	XXVII, XXVIII	13t, 5l, 4s, 3co
247	W, M, L	G	XXVII, XXVIII, XXIX	XXVII, XXVIII, XXIX	12t, 5l, 6s, 2co
418	B, M, L	G	XXVI, XXVII	XXVII, XXVIII, XXIX	Normal

pendent course (24 out of 94 instances). In the latter case a small anastomosing twig usually passes from the perineal branch to the main trunk of the posterior cutaneous. A separate perineal branch occurs much more frequently in the unusual forms of plexus than in the usual, as shown by the above table.

Frequently the perineal branch arises from the posterior cutaneous nerve in common with a large branch which passes to supply the medial surface of the leg (31 out of 110 instances). A branch of considerable size may arise in common with the perineal nerve and then pass upwards to be distributed over the medial margin of the gluteal muscle (15 out of 110 instances). Two separate perineal branches are given off from the posterior cutaneous nerve infrequently (5 out of 110 instances). Rarely the perineal branch gives off rami both for the buttock and for the medial surface of the leg (2 out of 110 instances).

Occasionally a root containing fibres destined mainly for the perineal nerve arises separately from the plexus, passes through the sacrotuberosal ligament and then joins the posterior cutaneous nerve immediately before this gives off the perineal branch (2 out of 94 instances).

Femoro-popliteal branches.—As a rule several branches arise from the posterior cutaneous nerve as it passes down the back of the thigh. Those on the medial side of the nerve are the better developed. In the most common form of distribution (67 out of 94 instances) three or four branches arise from the medial side of the nerve between where it emerges from under the gluteus maximus muscle and the popliteal space. On the lateral side two to three branches are commonly given off. Another common type of distribution is one in which the upper half or two-thirds of the medial posterior surface of the thigh is supplied by a branch which arises in common with the perineal branch of the posterior cutaneous nerve (24 out of 94 instances). This condition was found most frequently associated with an anterior type of plexus (type A, 1; type B, 4; type C, 9; type D, 5; type F, 1; type G, 1).

In one instance soon after the posterior cutaneous nerve emerged from under the gluteus maximus muscle a long medial branch arose to supply the inner side of the leg as far as the knee, and a long lateral branch to supply a corresponding lateral area. This was found on the right side of a subject with a normal, type D, plexus and a normal vertebral column. In another instance the posterior cutaneous after it emerged divided into two branches which extended to the knee and in addition a long medial branch arose from the perineal division of the nerve. This was found on the right side of a subject with a posterior, type G, form of plexus and 13 thoracic, 5 lumbar, 4 sacral and 3 coccygeal vertebræ. In one instance the upper medial portion of the thigh was supplied by a nerve arising from the perineal branch of the pudic nerve. This was found on the

right side of a subject with a normal, type D, plexus and a normal skeleton.

Terminal branches.—The absence of a posterior cutaneous nerve has been reported, but this condition I have not seen. In one instance (308-R) the terminal branches could not be followed as far as the knee by gross dissection. In the great majority of instances (81 out of 110) the terminal branches could be readily followed into the upper third of the back of the leg. But rarely was there found the branch described in Poirier and Charpy's anatomy as extending to anastomose with the sural nerve. In 21 out of 110 instances the chief terminal branch extended on the medial side of the leg well into the middle third of the back of the leg. In 8 instances out of 110 the chief terminal branch could be followed nearly to the medial malleolus. This extensive distribution of the posterior cutaneous nerve of the thigh was found twice associated with the B type of plexus, once with the C type, four times with the D type, and once with an F type. No obvious relation therefore exists between the extent of distribution of the posterior cutaneous nerve and the form of the plexus from which it springs. This also is true of relations to the origin of the nerve from the sacral plexus and to race, sex, and side of body.

2. Perforating Cutaneous Nerve.

A distinct perforating cutaneous nerve arising from the 2d and 3d sacral nerves and passing through the sacro-tuberosal ligament to supply the skin over the medial margin of the buttock was found in but 8 instances out of 94. To what extent this small percentage is to be attributed to lack of sufficient care in dissection cannot at present be stated. Only the better charts have been used in this tabulation. Eisler found the nerve in 22 out of 34 instances.

In one instance the perineal branch of the posterior cutaneous passed beneath the sacro-tuberosal ligament on the way to its destination.

3. Cutaneous Branches of the Peroneal Nerve.

a. N. Cutaneus surae lateralis.—This nerve arises in the popliteal space and runs down over the lateral head of the gastrocnemius to supply the lateral cutaneous area of the leg and usually sends a branch to anastomose with the n. cutaneus suræ medialis to form the sural (external saphenous) nerve. This form of distribution was found in 38 out of 76 instances. In 30 out of 76 instances the communicating branch to

the n. surae medialis was not found. In 7 out of 76 instances two branches arose from the peroneal nerve in the popliteal space. One of these supplied the side of the leg below the knee; the other supplied the back of the leg and sent a branch of communication to the n. surae medialis. This mode of distribution is described as the normal by many authors. In one instance the peroneal nerve gave rise to the sural (external saphenous) while the tibial furnished a cutaneous branch for the skin over the calf. In five other instances the n. cutaneus surae medialis gave rise to a cutaneous branch for the supply of the upper part of the calf. The extent of distribution of the various branches mentioned is inversely proportional to the extent of distribution of the posterior cutaneous nerve of the thigh and the saphenous and obturator nerves. Great individual variations are found.

b. N. Cutaneus peronei femoralis.—In one instance in which the tibial and peroneal nerves arose separately from the plexus the peroneal nerve passed between two divisions of the pyriformis muscle, then lateral to a fasciculus of the short head of the biceps which arose from the proximal end of the gluteal tuberosity. It crossed the antero-lateral surface of this fasciculus, and then between it and the main portion of the muscle to its usual position in the thigh. Near the middle of the shaft of the femur it gave off a branch which passed through the short head of the biceps to the side of the thigh where it divided into ascending and descending branches and supplied a large area between the territories of the n. cutaneus femoris lateralis and the n. cutaneus femoris posterior. This abnormal cutaneous nerve I have not found previously described. It resembles somewhat a nerve in the orang described by Klaatsch, **02.**

c. Nn. Cutanei dorsales pedis.—In the great majority of instances the n. cutaneus peroneus superficialis divides into two main terminal branches just above the ankle. One of these, the n. cutaneus dorsalis medialis, passes directly to the outer side of the big toe, giving off on its way a branch to supply the contiguous sides of the 2d and 3d toes and small branches to anastomose with those branches of the n. peroneus profundus which supply the contiguous sides of the first and second toes. The other, the n. cutaneus dorsalis intermedialis, passes down to supply the contiguous sides of the third and fourth, and fourth and fifth toes. This general mode of distribution was found in 44 instances out of 111 (about 40%). In two of the 44 cases above mentioned the sural nerve failed to extend to the little toe and a special

branch arose from the n. cutaneus dorsalis intermedialis to supply the outer side of the little toe. Variations in the cutaneous nerve supply of the dorsum of the foot occurred with the following frequency.

In 17 instances the n. cutaneus dorsalis lateralis (external saphenous) supplied the place of the n. cutaneus dorsalis intermedius and sent branches to the contiguous sides of the 4th and 5th, and 3d and 4th toes. In two instances a branch from the n. cutaneus dorsalis lateralis anastomosed with the n. cutaneus dorsalis intermedialis and the combined nerve then divided into branches for the contiguous sides of the 4th and 5th, 3d and 4th, and 2d and 3d toes. With the branch to the last a ramus from the n. cutaneus dorsalis medialis anastomosed. In 15 instances a branch from the n. cutaneus dorsalis lateralis anastomosed with one from the n. cutaneus dorsalis intermedius and the branches arising from the combined nerve supplied the contiguous sides of the 3d and 4th, and 4th and 5th toes. In two instances the n. cutaneus dorsalis lateralis sent a branch to anastomose with one from the n. cutaneus dorsalis intermedialis going to the 2d and 3d toes. In five instances the n. cutaneus dorsalis lateralis supplied the outer side of the little toe and the contiguous sides of the 4th and 5th toes. In 10 instances a branch from the n. cutaneus dorsalis lateralis anastomosed with the branch from the n. cutaneus dorsalis intermedialis sent to supply the contiguous sides of the 4th and 5th toes. In four instances the n. cutaneus dorsalis intermedialis supplied the contiguous sides of the 2d and 3d, 3d and 4th, and 4th and 5th toes while the n. cutaneus dorsalis medialis supplied the outer side of the first toe and aided in the supply of the contiguous sides of the 1st and 2d toes. In one of these instances the n. cutaneus dorsalis medialis arose in the leg from the n. peroneus profundus. In five other instances the nerve distribution was similar in nature but an anastomotic branch passed from the n. cutaneus dorsalis medialis to the nerve going to supply the contiguous sides of the 2d and 3d toes. In one of these instances the n. cutaneus dorsalis medialis arose from the n. peroneus profundus nerve and emerged from between the peroneus tertius and the extensor digitorum longus muscles. In two instances the n. cutaneus dorsalis medialis supplied, in addition to its usual territory, the contiguous sides of the 3d and 4th toes; the n. cutaneus dorsalis intermedialis was confined in distribution to the 4th and 5th toes. In one instance the n. peroneus profundus supplied in addition to its usual branches, the chief branches to the medial side of the great toe and the lateral side of the 2d toe.

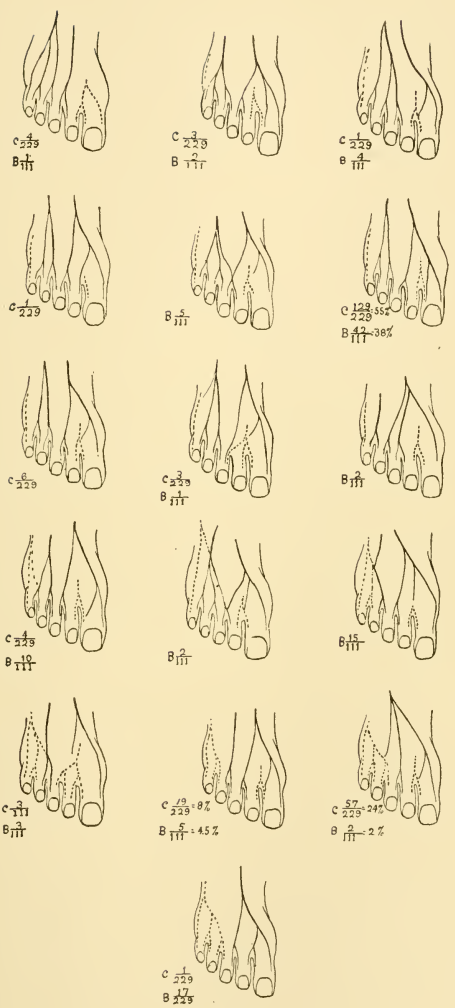


FIG. 1. The diagrams represent various types of distribution of the cutaneous nerves of the back of the foot. The frequency with which the various types of distribution were found by the Committee of Collective Investigation is indicated by *C* followed by a fraction of which the denominator represents the total number of feet tabulated; the frequency with which the various types occurred in the feet studied by the writer is indicated by *B* followed by a fraction of which the denominator is 111, the total number of feet tabulated.

In another instance it sent a branch to aid in the supply of the contiguous sides of the 2d and 3d toes and in three instances it formed the chief source of supply for the contiguous sides of the 1st and 2d, and 2d and 3d toes.

In two instances, as we have seen above, the n. cutaneus dorsalis medialis arose in the leg from the n. peroneus profundus. In five instances the n. cutaneus dorsalis medialis arose from the peroneal nerve soon after this passed beneath the head of the peroneus longus muscle. The nerve then took a course somewhat independent of that of the n. cutaneus dorsalis intermedialis.

The very great variation found in the distribution of the nerves of the dorsum of the foot seems not to be associated with such factors as age, sex, race, side of body or relative position of the lumbo-sacral plexus. Rough estimation of these factors have given so little promise of positive results that I omit here a detailed tabulation.

For the sake of comparison the following data obtained by the Committee of Collective Investigation of Great Britain and Ireland[4] are appended. Our results agree with regard to the condition most frequently observed, although I found this condition in but 40% of the feet examined while the committee found it in 55%. In those instances in which the n. cutaneus dorsalis lateralis served wholly or in part to supply the contiguous sides of the 3d and 4th, and 4th and 5th toes, although the total frequency is approximately the same there is considerable difference in frequency in variation in the nature of the relations between the n. cutaneus dorsalis intermedialis and lateralis.

4. Cutaneous Branches of the Tibial Nerve.

The nearly constant origin of the chief root of the n. suralis from the tibial nerve and the account of it which I have given above in treating of the cutaneous branches of the peroneal nerve render further description here unnecessary. The rami calcanei mediales vary somewhat in extent of distribution but offer no features of special interest. The cutaneous supply of the toes is singularly constant, the main variation being found in the extent of development of a branch from the n. plantaris medialis to the nerve supplying the 4th and 5th toes, or from the n. plantaris lateralis to the nerve supplying the 3d and 4th toes.

In 69 out of 87 instances no well developed branch of this nature

[4] Journal of Anatomy and Physiology, Vo. 26, 1892, p. 89.

was found. In eight instances a branch passed from the n. plantaris medialis to the nerve to the 4th and 5th toes. In seven instances a branch passed from the n. plantaris lateralis to the nerve supplying the contiguous sides of the 3d and 4th toes. In two instances the n. plantaris lateralis furnished the chief supply of the contiguous sides of the 3d and 4th as well as of the 4th and 5th toes. In one instance the n. plantaris medialis supplied the contiguous sides of all the toes.

VI. CUTANEOUS BRANCHES TO THE INFERIOR EXTREMITY FROM THE DORSAL DIVISIONS OF THE SPINAL NERVES.

In Embryo CXLIV, length 14 mm. (Plate IV, Fig. 2), the lateral branches of the dorsal divisions of the last five or six thoracic and the first three lumbar nerves may be followed to the subcutaneous tissue. They take at this period a somewhat simple course and have a distinctly segmental arrangement. The dorsal divisions of the fourth and fifth lumbar and of the sacral and coccygeal nerves are connected by anastomosing branches. From these nerves rami may be followed toward, but cannot be followed distinctly into the skin.

In Embryo XXII, length 20 mm. (Plate V, Fig. 1), the lateral branches of the first three lumbar nerves may be followed distally to the base of the limb where they terminate over the proximal margin of the iliac crest. The branches of the second and third lumbar nerves are connected at this period by anastomoses, although the plexiform arrangement characteristic of the adult is not yet apparent. In subsequent development these nerves extend over the postero-lateral surface of the thigh, nn. clunium superiores. For the variations in origin of these nerves in the adult, see Bardeen and Elting, 01.

The lateral branches which arise from the dorsal divisions of the first three sacral nerves anastomose and from them in Embryo XXII two delicate branches may be traced toward the skin, nn. clunium mediales. There is considerable variation in these nerves in the adult, but I have not sufficient data on which to base a statistical study of the subject.

VII. SUMMARY AND GENERAL CONCLUSIONS.

The cutaneous nerves of the posterior limb in the embryo first approach the anterior, posterior and distal margin of the limb-bud and from these areas send branches of distribution over the medial (ventral) and lateral (dorsal) surfaces of the developing limb. This method of development may be recognized in the adult. The chief nerve trunks approach the fascia in a line which corresponds fairly closely with the primary margins of the limb. The posterior cutaneous

nerve of the thigh and the sural nerve are both shifted over the dorsal (primary medial) surface of the thigh during development, but none the less distribute their cutaneous branches from a line which corresponds to some extent to the posterior margin of the limb-bud. The line along which the anterior cutaneous nerves of the thigh reach the fascia likewise corresponds with the original anterior margin of the limb-bud. In Plate VII, Figs. 1 and 2, a schematic diagram is given to illustrate the mode of distribution of the cutaneous nerves of the adult limb. So far as possible each main nerve is represented approximately as it occurs with the greatest frequency.

This mode of distribution of the cutaneous nerves from the region of the margin of the limb-bud is probably due to a differentiation of function, the musculature of the limb-bud being differentiated on the medial and lateral surfaces and the margins serving for the primary development of the cutaneous areas. In sharks the cutaneous branches supplied to the dorsal and ventral surface of the fin extend upwards in numerous branches between the muscle bundles. In most higher forms a distribution of cutaneous nerves from the margins of the limb is well marked, although numerous exceptions occur.

It is of interest to inquire whether or not anything may be found during embryonic development to account for the segmental distribution of the nerves of the limb described on the basis of physiological and clinical evidence by Head, Sherrington, Bolk and a large number of other investigators. It is quite certain that no evident dermatomes associated with specific spinal nerves are to be found in the embryo. It seems probable that the cutaneous nerve fibres contained in a given spinal nerve find a path of least resistance toward the marginal area lying most directly opposite and that to any given area one or two nerves may thus serve to furnish the bulk of the fibres. In Figs. 2 and 3 I have shown diagrammatically the approximate marginal areas to which each spinal nerve most directly contributes in the embryo. Subsequently these areas become extended by the growth of branches from the margins of the limb over the medial and lateral surfaces.

The great variation in the distribution of the nerves supplied to different areas can be best accounted for, I think, by assuming that the nerves grow as plants grow: in part they are guided in their course by definite paths, as climbing plants may be guided by strings, but where definite paths are not offered great variation in the distribution of the cutaneous rami may be seen. Extensive development of one nerve tends to retard its neighbors, lack of development tends to excite them to more active growth.

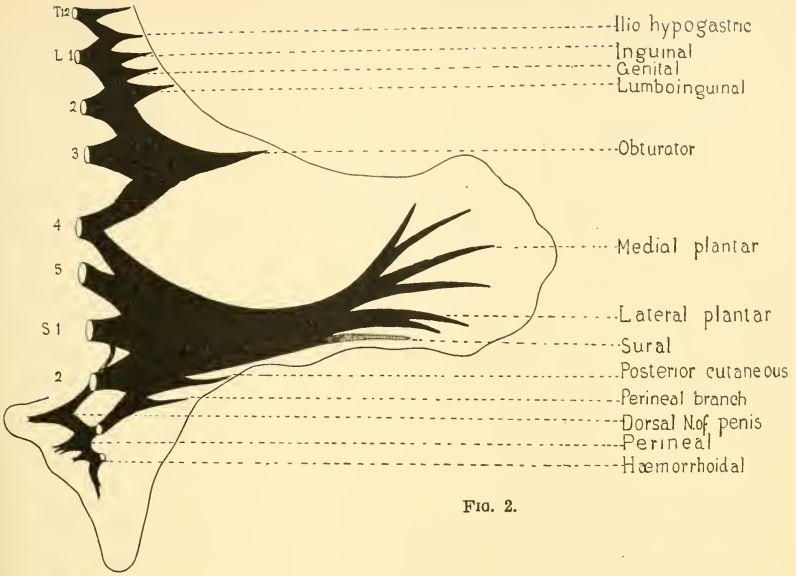


FIG. 2.

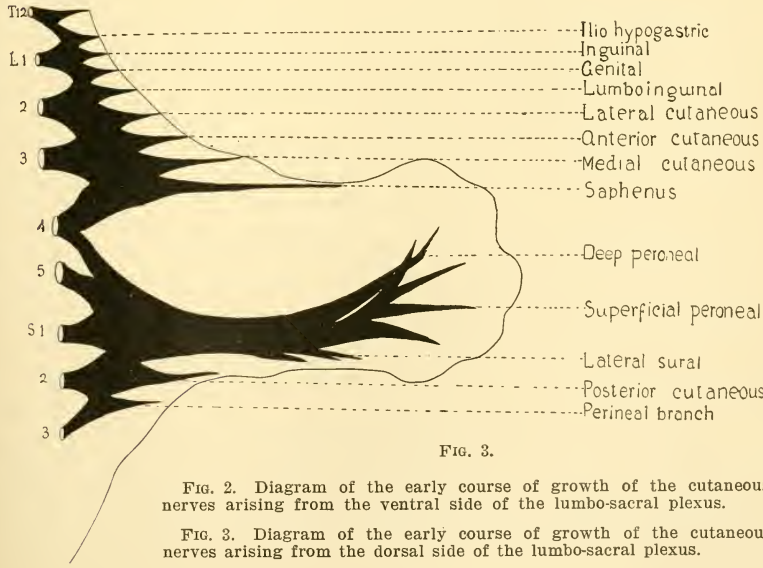


FIG. 3.

FIG. 2. Diagram of the early course of growth of the cutaneous nerves arising from the ventral side of the lumbo-sacral plexus.

FIG. 3. Diagram of the early course of growth of the cutaneous nerves arising from the dorsal side of the lumbo-sacral plexus.

C. DEVELOPMENT OF THE MUSCULATURE AND DEVELOPMENT AND VARIATION IN DISTRIBUTION OF THE NERVES TO THE MUSCLES OF THE INFERIOR EXTREMITY.

I. FEMORAL GROUP.

a. Embryonic Development.

1. General Features.

Soon after the femoral nerve begins to extend into the base of the limb differentiation of the femoral musculature commences. At about the center of the shaft of the femur a mass of tissue may be distinguished as the anlage of the quadriceps muscle (Plate III, Fig. 2). Into a cleft in this tissue the main trunk of the femoral nerve extends and gives off branches for each of the main divisions of the quadriceps. Anterior to this an ill defined mass of tissue may be distinguished as the anlage of the sartorius muscle. A special nerve is given to this. About the main trunk of the nerve as it passes over the region of the acetabulum a mass of tissue represents the anlage of the iliopsoas and possibly also the pectineus muscles. In this region the lateral and anterior cutaneous nerves of the thigh pass toward the ectoderm. Distalward the anlage of the saphenous nerve may be seen.

In a slightly older embryo (Plate VI, Fig. 1) the muscle differentiation is much further advanced. The sartorius muscle extends well toward the blastema of the ilium and toward the medial surface of the proximal end of the tibia. Definite tendons are not, however, developed. The nerve to the sartorius extends a short distance distally within the substance of the muscle. The iliopsoas muscle is likewise further differentiated and has extended more toward the vertebral column. The pectineus muscle has become distinct and to it runs a branch from the femoral nerve.

The quadriceps muscle begins to show definite differentiation. Tendons of attachment are not, however, clearly differentiated. The various branches of the nerve to this muscle, Fig. a, have extended further into the body of the muscle. They follow in this course developing lines of cleavage of the muscle into its constituted portions.

The various cutaneous nerves mentioned above have extended considerably in length and in addition there are two medial cutaneous branches. These run toward the skin in the dense fascia which now separates the obturator from the femoral group of musculature.

In an embryo of 20 mm. (Plate VI. Figs. 2 and b) the individual muscles of this group are clearly demarkated. The figures illustrate

sufficiently clearly the position of the various nerves and muscles. The muscles are all attached to the skeletal apparatus by distinct tendons. The main nerve trunks run in the connective tissue which serves to separate the various muscles from one another and to divide each muscle into its constituent parts.

2. Individual Muscles.

Iliopsoas muscle (Plate VI, Figs. 1 and 2). The iliopsoas muscle arises from a mass of tissue which embraces the femoral nerve as it passes into the limb-bud. In subsequent development the iliacus muscle spreads out over the ilium, the psoas major muscle extends up along the course of the roots of the femoral nerve to form its attachments to the vertebral column, and in close union the two muscles extend distally to be attached to the lesser trochanter. The psoas minor seems to be differentiated from the psoas major muscle anlage, but this is uncertain.

To the psoas major as it is developed toward the vertebral column branches are given from the femoral nerve or its roots of origin as far anterior as the 22d spinal (2d lumbar) and occasionally as far as the 21st spinal nerve. These branches extend in between the developing bundles of the muscle and have a complex, extensive distribution.

The nerve to the psoas minor muscle frequently arises from the trunk of the genito-femoral or from the lumbo-inguinal branch of this nerve.

To the iliacus muscle as it spreads out over the surface of the ilium several branches, often united in a plexiform manner, are given. These branches pass across or near the superficial surface of the muscle about midway between the crest of the ilium and the combined iliopsoas tendon. Special nerve branches are likewise usually distributed from the main trunk of the femoral nerve to the fleshy portion of the muscle as it passes over the acetabulum and the head of the femur.

There is considerable variation in the exact mode of distribution of the nerves mentioned. Frequently a special layer of the iliacus covers the nerves distributed to this muscle. The trunk of the femoral nerve may be divided by one or more bundles of the iliopsoas muscle. The variations in the distribution of the nerves to the iliopsoas muscle do not readily lend themselves to statistical treatment and hence this is here omitted.

The iliopsoas is probably represented in the urodela and reptiles by the posterior portion of the pubi-ischio-femoralis internus and the anterior margin of the ilio-femoralis. The psoas muscle, which is phylogenetically younger than the iliacus, is by many (see Pardi, 02) considered to be a prevertebral muscle belonging primitively to the trunk musculature. Its ontogenetic de-

velopment from an anlage common to it and the iliacus muscle indicates that it should be placed with the intrinsic musculature of the limb.

The phylogenetic development of the psoas minor, on the other hand, is somewhat uncertain. It may be derived from the trunk musculature. This is perhaps indicated by its frequent innervation through a branch from the genitofemoral nerve, while the psoas major is innervated by branches which arise from the femoral nerve or its roots. Its embryonic origin should be studied in some of those forms in which the adult muscle is highly developed.

In mammals with an ilium triangular in cross-section the iliacus lies externally, a position which corresponds with the situation in which its anlage appears in the human embryo (Lubsen). In those forms in which the iliac blade is developed the muscle comes to have an internal position.

The variations of the iliopsoas in man are chiefly those of a greater or less independence of the two muscles composing it and a greater or less specialization of fasciculi in either. There are also slight variations in the origin and attachment of the muscles. The very inconstant psoas minor varies chiefly in the extent of its development. The inferior insertion may take place into the iliac fascia, the inguinal ligament, the femur between the small trochanter and the head, or together with the iliopsoas into the small trochanter (Le Double). Fasciculi may unite the psoas major and the psoas minor. These variations may indicate a common origin of the two muscles.

Pectineus (Plate VI, Figs. 1 and 2). In an embryo 11 mm. long the anlage of the pectineus is not distinct. It may be represented in those portions of the iliopsoas and the obturator muscle anlages which lie nearest the region in which the pectineus will be developed. Gräfenberg, 04, describes in the region immediately distal to the superior pubic ramus a union of a branch of the obturator nerve with a branch of the femoral before the muscle anlage of the pectineus appears. This I have not found in the embryos of a corresponding stage which I have examined. Gräfenberg describes the pectineus anlage when it first appears as fused proximally with the iliopsoas anlage. It is probable that the superficial portion of the pectineus is thus at one stage usually fused with the iliopsoas anlage. In the youngest embryo in which I have found it distinct it is, however, separated by a small interval from the iliopsoas muscle mass (Plate II, Fig. 3) and seems more closely associated with the anlage of the adductor longus.

In this 14 mm. embryo (Plate VI, Fig. 1) the anlage of the muscle is closely applied to the pubic blastema and can be followed from the body of the pubis to the blastema of the femur. Into it a nerve branch may be traced from the femoral nerve. Dorsal to the obturator nerve in the obturator foramen is a mass of tissue closely associated with the anlage of the obturator externus on the one side and with that of the pectineus on the other. No definite nerve branch can be traced into it from the

obturator nerve, but it seems not improbable that this represents the anlage of that portion of the pectineus which is supplied by the obturator nerve in many individuals.

In an embryo of 20 mm. (Plate VI, Fig. 2), the pectineus occupies a position which corresponds with that of the adult muscle. The femoral nerve gives to it a large branch which passes at first across its outer surface about midway between its tendons of origin and insertion. I have been unable to trace a branch to it from the obturator nerve.

In the adult, as is well known, the nerve supply of the pectineus is usually through a branch from the femoral but not infrequently also from the obturator or the accessory obturator nerve. Paterson (91, 95) has shown that in man the muscle is often divisible into a superficial portion supplied by the femoral nerve and a deep portion supplied by the obturator nerve. Similar conditions are normal in some of the lower mammals, while in others the muscle may be supplied by the femoral nerve only or the obturator nerve only. The dorsal or femoral portion is probably derived from an anlage intimately associated with the iliopsoas anlage, the ventral portion from the anlage of the obturator externus. W. Leche has shown that in many mammals there is separated from the obturator externus a muscle which he calls the obturator intermedius and that in those forms in which the pectineus is supplied by the obturator nerve it is probable that this obturator intermedius has entered into the formation of the anlage of the pectineus.

The pectineus of the mammals is, together with the iliopsoas, probably represented in the urodeles and reptiles by the pubo-ischio-femoralis internus. This, like the pectineus, may be supplied wholly by the femoral, or in part also by branches from the obturator. The accessory obturator nerve, which in about 10% of bodies innervates, or helps to innervate, the pectineus muscle in man, indicates, perhaps, that the division of the limb musculature in man into dorsal and ventral portions is not strictly to be traced in the respective territories of the femoral and obturator nerves. Nerve elements belonging to the ventral territory may be normally bound up in the femoral nerve in those branches which supply the pectineus muscle. When those branches become isolated we have the accessory obturator nerve (see Eisler, 92).

There is considerable variation in the extent of separation of the pectineus into two portions in the human body. The muscle is very frequently fused with the adductor longus. Occasionally a fasciculus passes from the iliacus to the pectineus or between the pectineus and the obturator externus.

Sartorius.—The sartorius develops from an anlage not directly fused with that of the quadriceps. Gräfenberg has described a fusion near the ilium of the proximal ends of the anlages of the rectus muscle and the sartorius with that of the iliacus. In those embryos I have studied in which these anlages are beginning to appear the quadriceps anlage is quite distinct from that of both the sartorius and iliacus. The upper limit of the sartorius anlage approaches closely, however, the iliacus

anlage. The first well marked differentiation of the sartorius anlage takes place in a region corresponding with that in which the nerves enter the muscle in the adult, Plate VI, Fig. 1. From here the differentiation of the muscle extends towards its iliac and tibial attachments. The embryonic muscle is proportionately larger than the adult muscle and forms more extensive tibial attachment. (Plate VI, Fig. 2, Plate VIII, Fig. 2).

Simultaneously with the differentiation of the muscle, branches extend into it from the femoral nerve. These branches are more or less intimately bound up with the anterior (middle) cutaneous nerve. As a rule there are two main branches, one of which serves to supply chiefly the lateral and proximal, the other the distal and medial, portion of the muscle. For the distribution of branches in the adult, see Frohse, 98.

In the urodeles the sartorius does not seem to be represented. In reptiles it is probably represented by the ambiens or the pubi-tibialis or both. The ambiens arises either from the ilium, as in the crocodile, or from the pubis, as in most forms. Its tendon passes to the front of the leg. It is an extensor of the knee. The tendon of the pubi-tibialis passes to the back of the leg. It is a flexor of the knee (Gadow, 82). It is probably not homologous with the pubi-tibialis of urodeles, which is innervated by the sciatic nerve and is a differentiated portion of the pubi-ischio-tibialis.

In the monotremes and insectivora the proximal attachment of the sartorius is in the neighborhood of the ilio-pectineal eminence. In the marsupials, prosimians, and primates it takes place into the ventral margin of the ilium. In other mammals it may take place in either place or from an intermediate region, from the ilio-pectineal fascia, the tendon of the psoas minor, the inguinal ligament, etc. (W. Leche). The insertion takes place into the medial side of the tibia or into the crural fascia. It may be fused with the gracilis at its insertion. It may be double (dog—Ellenberger and Baum). The partial longitudinal splitting of the muscle found in the dog and other carnivora and as a variation in man may possibly indicate a primitive relationship to two muscles, the ambiens and the pubi-tibialis of reptiles. There seems to be nothing either in the phylogenetic or the ontogenetic history of the muscle to account for the transverse tendinous inscription or tendon which occasionally is found dividing the muscle into two parts.

Quadriceps Femoris Muscle, Plate VI. *Rectus Femoris.*—This muscle is developed from the quadriceps muscle mass by gradual differentiation. Its tendon of attachment to the anterior inferior iliac spine is developed later than that to the supra-acetabular groove, Roger Williams, 78, and is a consequence of the development of the iliac blade, Le Double, 97. As a rule the nerve to the muscle divides into two main branches, one of which goes chiefly to the medial half, the other chiefly to the lateral half of the muscle. The main trunk of the former, which enters about a

third of the distance from the anterior extremity of the muscle, may be followed for a considerable distance toward the distal extremity of the muscle; the main trunk of the latter, which enters more anteriorly, extends a less distance distally and has a recurrent branch which extends toward the proximal extremity of the muscle. This branch has been followed to the iliac insertion of the muscle and has been reported as extending to the M tensor fasciæ latæ. This last condition I have never seen.

Vastus Lateralis.—This muscle is differentiated from the quadriceps muscle mass by the development of septa between it and the vastus intermedius. The muscle is usually composed of two distinct layers. an outer and an inner, separated by fascia containing nerves and blood vessels. Often the inner layer is further partially subdivided into two sheets by fascia in which nerves and blood vessels run. Commonly the nerve to the vastus lateralis divides into three branches of which one runs on the inner surface of the outer layer of the muscle, the second betwen the two sheets of the inner layer, and the third passes through the inner sheet of the inner layer to be distributed to the most lateral part of the vastus intermedius muscle. The larger intrinsic nerve trunks cross the fasciculi of the muscle sheets and about midway between the extremities of the fasciculi.

Vastus Intermedius.—This is differentiated from the quadriceps muscle mass at the time of the ingrowth of the main nerves and blood vessels of the muscle. The muscle is composed of muscle lamellæ concentrically arranged about the diaphysis of the femur. The lowest, most distal, and most completely separated of these lamellæ is the subcrureus muscle. Several nerves are distributed to the muscle. To the lateral region a branch from the nerve to the vastus lateralis usually extends. A special ramus from the femoral nerve generally passes to the middle portion, and from the nerve to the vastus medialis several branches are often given to the medial side of the muscle.

Vastus Medialis.—This muscle is differentiated from the quadriceps muscle mass by the formation of a connective tissue sheet between it and the vastus intermedius. Its nerve of supply runs along on the medial surface of the muscle sending branches from time to time into its substance and finally near Hunter's canal the terminal twigs of the nerve enter the muscle. The nerve is often more or less bound up with the saphenous nerve. The rami which enter the muscle extend at first across the fibre-bundles of the muscle sheet about midway between the extremities of the fibre-bundles.

The rectus is phylogenetically the oldest part of the quadriceps. In the urodela, where it is represented by that part of the ilio-tibialis supplied by the femoral nerve, there seem to be no muscles which correspond with the vasti but these are differentiated in the reptiles and the higher forms. In some of the mammals the three vasti of the muscle are more or less fused. In man the chief variations found in the quadriceps result from a greater or less division of the primitive extensor mass into individualized parts.

b. Nerve Variation.

1. Variation in Origin of the Femoral Nerve.

The femoral nerve in the great majority of instances arises in the main from the 22d, 23d, and 24th spinal (2d, 3d, and 4th lumbar) nerves. There is, however, considerable variation in the size of the nerve bundles derived from the 22d and 24th spinal nerves. The 21st spinal nerve usually contributes some fibres, and the 20th and 25th spinal nerves occasionally do so. In Table XIV there is shown the frequency with which certain root origins of the femoral nerve were found and the relation of these various modes of origin to various types of plexuses. A study of these variations in relation to race, sex, and side of body has revealed no marked associations, and therefore the tables embracing these data are omitted.

2. Relations of the Branches Springing from the Femoral Nerve to the Nerve Roots.

In considering the cutaneous nerves we have seen reason to believe that the peripheral segmental distribution of the spinal nerves disclosed by physiological experiments, is due to a directness of growth which a given spinal nerve has toward a given peripheral area so that the nerve can send more fibres into this area than its neighbors can and hence serves in the main to innervate the area. This is also probably true of muscle innervation. In Plate III, Fig. 3, is shown the position of the femoral nerve as it enters the femoral muscle mass in a young embryo. The following diagram shows a cross section of the adult femoral nerve as it passes under the inguinal ligament. The regions occupied by the fibres of the chief motor and sensory branches are outlined, while the approximate areas occupied by the main bulk of the fibres of each spinal nerve are shown by stippling the position occupied by the fibres of the 23d spinal (3d lumbar) nerve. While the diagram is schematic it may serve to illustrate the relation of peripheral to spinal nerves of the femoral

TABLE XIV.

Type of Plexus from which N. Femoralis arises:		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Frequency of Origin of N. Femoralis from:						
Type.				Nn. Sp. [XX][5] XXI XXII XXIII XXIV	Nn. Sp. [XX] (XXI) XXII XXIII XXIV	Nn. Sp. [XXI] (XXII) XXIII XXIV	Nn. Sp. (XXII) XXIII XXIV	Nn. Sp. [XXI] (XXII) XXIII XXIV XXV	Nn. Sp. (XXII) XXIII XXIV XXV	Total Number.
	A	XXIV	XXVI	1						1
Ant.	B	XXIV	XXVII		15	5	5			25
	C	XXIV chiefly to sacral plexus	XXVIII	2	42	10	9			63
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII	28		50	26			104
	E	XXIV-XXV or XXV	XXVIII					6	12	18
Post.	F	XXIV	XXIX	3		3	9			15
	G	XXIV-XXV or XXV	XXIX					4	16	20
Total Number.....				34	57	68	49	10	28	246

[5] The nerves enclosed in brackets contribute fibres to the N. cut. fem. lat. but probably not to other branches of the N. femoralis; those enclosed in parentheses contribute few fibres to the N. femoralis.

group. A truly accurate determination could be made only by sectioning each of the various roots and each of the various nerves of distribution in various plexuses and then following the paths of degeneration.

3. Variation in the Association of the Terminal Branches Arising from the Femoral Nerve.

Soon after the femoral nerve passes under the inguinal ligament it gives rise to the various branches which serve to innervate the skin and muscles of the front of the thigh and the arteries and joints. There

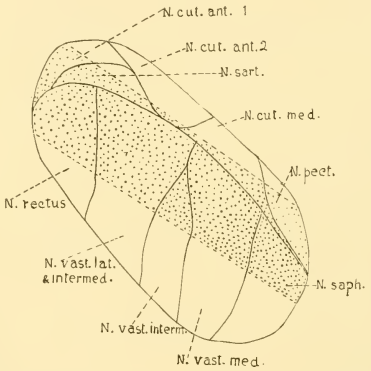


Fig. 4. Diagram to illustrate the approximate regions of the femoral nerve trunk occupied by the fibers which pass out into its muscular and cutaneous branches. The stippled area indicates the position of the main bulk of the fibers of the third lumbar nerve.

is great variation in the association of the nerves going to these various structures. Now one set of nerves may be bound in a common trunk for a part of their course, now another. The association of the various nerves into common trunks is limited, however, to the association of contiguous regions shown in the diagram, Fig. 4. Two or more of these regions may continue for a time to be associated after the femoral trunk has split into branches. Thus the nerves of the rectus femoris muscle are often bound for a part of their course with those of the vastus lateralis or with those of the vastus lateralis and intermedius before the final division takes place. But the nerve to the rectus femoris muscle is never found bound up in a common trunk with the nerve to the vastus medialis or the saphenous nerve unless the nerves to the vastus lateralis and

vastus intermedius are also included in the trunk. In the following table the association of nerve branches found in 77 instances is indicated, and at the left is shown the number of times each condition was found. Minor differences are not recognized and no attempt has been made to include the nerves for the arteries and joints. In one instance the femoral nerve was found to furnish a branch to the adductor longus muscle. This is said by Poirier to be a normal condition.

TABLE XV.

Association of the Branches of Distribution of the Femoral Nerve in Common Nerve Trunks.

A. No distinct division into two parts.

1	[P] [(S1, S2, S1) (S3, A2) (I)] [(R1, R2) (L1, L2, L3) (C1, C2) (M) (Sph)]
7	[P] [I] [(A1) (A2)] [(S1, S2)] [(R1) (R2) (L1, L2, L3) (C1, C2)] [(M) (Sph)]
1	[a] [(P) (I)] [(S1, S2) (A2)] [(R1, R2) (L1, L2, L3) (C1, C2)] [(M) (Sph)]
1	[P] [(A1) (A2) (S2)] [I] [(R1, R2) (L1, L2, L3) (C1, C2)] [M] [Sph]
1	[X[6]] [(A2, S1) (S2) (I1) (I2)] [(R1, R2) (L1, L2, L3) (C1, C2)] [M] [Sph]
2	[P] [A1, A2, S1, S2, S3] [(R1, R2) (L1, L2) (C1) (C2)] [M] [(Sph) (I)]
3	[P] [(A1, S2) (S2, S3)] [(I1) (I2) (I3)] [R1, R2] [L1, L2, L3] [C1, C2] [M1, M2, M3] [Sph]
1	[P] [I] [S1] [A, S2] [(R1, L1) (R2, L2)] [L3, C1, C2] [M] [Sph]
1	[P] [S1, S2, A] [R1, R2, L1] [C1, C2, M] [I] [Sph]
1	[?] [S1, S2, A1, A2] [(R1, R2) (L1, L2)] [L3] [C1, C2] [M] [Sph, I]
1	[?] [(A1) (S1, S2) (A2, S3)] [(R1, R2) (L1, L2)] [C1, C2] [M1, M2] [Sph, I]
1	[(P) (branch to adductor longus)] [I] [S1, S2, A1, A2] [(R1, R2) (C1, C2) (L1, L2, L3)] [(M) (Sph) (I)]
1	[P1, P2] [(S1) (A, S2, S3) (I)] [(R1, R2) (L1, L2, L3)] [C] [(M) (Sph)]
7	[P] [(A1, S1, A2, S2) (I)] [R1, R2] [(L1, L2, L3) (C1)] [(C2) (M)] [Sph]
4	[(P1, P2) (I)] [S1, S2, A1, A2] [R1, R2] [(L1, L2, L3) (C)] [(M) (Sph)]
1	[X] [S1, S2, A1] [I] [R1, R2, R3] [(L1, L2, L3) (C1, C2)] [(M) (Sph)]
2	[X] [P] [S1, S2, A1, A2] [R1, R2] [(L1, L2) (C)] [M] [(Sph) (I)]
4	[P] [(S1, S2) (A1)] [R1, R2] [L1, L2, L3] [C1, C2] [(M) (Sph) (I)]

[6] From the 23d spinal nerve.

1 [a] [P] [S1, S2] [A] [R1, R2] [(L1, L2, L3) (C1, C2)] [(M) (Sph) (I)]
3 [P] [I] [A1] [S1, S2, A2] [R1, R2, R3] [L1, L2, L3, C1] [(M) (C2) (Sph)]
4 [P1, P2] [I] [S1, S2, A1, A2] [R1, R2] [L1, L2, L3] [C1, C2] [(M) (Sph)]
2 [a] [P] [I] [(S1, S2) (A2)] [R1, R2] [L1, L2, L3] [C1, C2] [(M) (Sph)]
1 [X] [a] [(S1, S2) (A2)] [(L) (I2)] [R1, R2] [L1, L2, L3] [C1, C2] [M] [Sph]
1 [P] [I] [S1, S2, A1] [R1, R2, R3] [L1, L2, L3] [(M1, M2) (C1, C2)] [Sph]
3 [P] [(S1, S2) (A)] [R1, R2] [L1, L2] [C1, C2] [M] [(Sph) (I)]
1 [P] [I] [(S1, S2) (A1, A2) (R1, R2)] [L1, L2] [(C) (M)] [Sph]
11 [P] [I] [S1, S2, A1, A2] [R1, R2] [L1, L2, L3] [C1, C2] [M] [Sph]
3 [a] [P] [S1, S2, A2] [I] [R1, R2] [L1, L2, L3] [C1, C2] [M] [Sph]
2 [a] [X] [P] [S1, S2, A2] [I] [R1, R2] [L1, L2, L3] [C1, C2] [M] [Sph]
1 [X] [(S1, S2) (S1, A1)] [R1, R2] [L1, L2, L3] [C1, C2] [M] [Sph]

B. Distinct division of femoral nerve into two parts.

1 I. [(P) (I)] [(S1, S2) (A1, A2)] [R1, R2, R3] [L1, L2] [C1] [C2]
II. [C3] [M] [(Sph) (I)]
1 I. [P] [S1, S2, A1, A2] [S3] [C1, C2] [M] [(Sph) (I)]
II. [R1] [R2] [L1, L2]
1 I. [P] [I] [A1, A2] [L2, L3] [M] [Sph]
II. [S1, S2] [R1, R2] [L1, L2]
1 I. [a] [P] [I] [(S1) (S2) (A2)]
II. [S2] [R1, R2] [L1, L2] [C1, C2] [(M1, M2, M3) (Sph)]
1 I. [P] [S1, S2] [(A1) (A2)] [S3] [(I1) (I2)]
II. [(R1) (R2)] [(L1) (L2)] [M1, M2] [Sph]

P—Nerve to pectineus muscle.
S—Nerve to sartorius muscle.
R—Nerve to rectus femoris muscle.
L—Nerve to vastus lateralis muscle.
C—Nerve to vastus intermedius muscle.
M—Nerve to vastus medialis muscle.
A—Anterior cutaneous nerve.
a—Anterior cutaneous nerve arising directly from plexus.
I—Medial cutaneous nerve.
S—Saphenous nerve.
X—Accessory obturator nerve.

In this table the various muscular and cutaneous nerves are represented by letters. The numerals placed after a letter indicate a first, second, or third nerve. The nerve trunks arising directly from the femoral are enclosed in brackets. The component nerves which are bound up in a common trunk for but a short distance are enclosed in parentheses. Those

which are united for a longer distance are separated by commas. In several instances the femoral nerve divided into two main divisions before separating into branches. These are shown in part B of the table.

II. OBTURATOR GROUP.

a. Embryonic Development.

1. General Features.

In an embryo 11 mm. long (Plate III, Fig. 1), the obturator nerve passes about the pelvic blastema between the pubic and ischial process and terminates some distance beyond in several branches. Differentiation of musculature is beginning in the region about the terminus of the nerve. One branch of the nerve terminates in a mass of tissue which represents the anlage of the obturator portion of the M. adductor magnus, and possibly also the M. obturator externus. The main nerve trunk then breaks up into several short branches about which lies a mass of tissue representing the anlage of the adductor longus and brevis and the gracilis muscles

In an embryo of 14 mm. (Plate VIII, Fig. 1) the individual muscles may be clearly recognized. None of them have well developed tendons. The figure represents sufficiently well the relations of the adductor muscles at the period under consideration. The gracilis muscle is merely outlined in order to show the short and long abductors. Each muscle is separated from its neighbors by a loose connective tissue. In this tissue the nerves take their course to the muscles. The nerve to each muscle strikes it about the center of greatest development, and may extend into the muscle substance for some distance. The paths for this intramuscular nerve growth are not in all cases clearly marked. In older embryos they are much plainer. The obturator and sciatic portions of the adductor magnus muscle are distinctly separate. See also Plate II, Fig. 3.

In an embryo of 20 mm. (Plate VIII, Fig. 2) the muscles have all become attached by tendons to the skeleton. Merely the origins and attachments of the adductor brevis and gracilis muscles are shown in Fig. 2. Figs. a and b represent the gracilis and adductor brevis muscles seen from the deep surface. The obturator and sciatic portions of the adductor magnus muscle have become fused.

In slightly older embryos the muscles become much more separated by relatively great development of intermuscular connective tissue than is found in late fœtal life and after birth. Compare Figs. 2, 3, and 4, Plate II, with figures of frozen sections of the adult limb.

The cutaneous branch of the obturator nerve is not clearly distinguishable in the embryos studied. In embryo XXII the articular branch of the nerve to the adductor magnus muscle is well marked.

2. Individual Muscles.

Gracilis.—The anlage of this muscle becomes distinctly differentiated at an early stage (Plate II, Fig. 3; Plate VIII, Figs. 1, 2, and a). It first appears in a region which corresponds with that in which the nerve enters the deep surface of the muscle in the adult, near the junction of the proximal with the middle thirds. From this region the muscle extends toward its pubic and tibial attachments.

In urodeles and reptiles (Saurians) the place of the gracilis is taken by the pubi-ischio-tibialis, which is innervated by the tibial portion of the sciatic nerve in the former and by the sciatic and obturator nerves in the latter (Gadow, 82). In all the mammals it is innervated by the obturator nerve. In several mammals the origin takes place from the abdominal wall anterior to the pubis. The insertion is usually in considerable part into the crural fascia and in some forms (edentates) extends to the foot (W. Leche). In many mammals the gracilis near its insertion is fused with the sartorius. Origin by two heads and fusion near the insertion with the sartorius have been found as variations in man. On the whole, however, the muscle is singularly independent.

Adductor brevis. Plate II, Fig. 3; Plate VIII, Figs. 1, 2, and b. This muscle is differentiated at first in somewhat close association with the obturator externus muscle and with the obturator portion of the adductor magnus. From its anlage processes of attachment are sent toward the pubis and femur (Plate VIII, Fig. 2). In the adult the muscle is usually innervated by a nerve which enters its middle third near the proximal border.

Adductor longus. Plate II, Fig. 3; Plate VIII, Figs. 1 and 2. This is differentiated from a muscle mass at first not perfectly distinct from that of the adductor brevis and in a region corresponding with that where the nerves enter the muscle. From here the muscle extends to its attachments. In the adult the nerve usually enters the deep surface of the muscle in several branches about midway between its tendons of origin and insertion.

Obturator externus. Plate II, Fig. 3; Plate VIII, Figs. 1 and 2. This muscle is differentiated from dense tissue lying beneath the obturator nerve in the obturator foramen and close to the embryonic hip joint. From here it extends to its attachment to the femur. The relations of the obturator externus to the pectineus muscle have been described

above, p. 302. The nerve for the obturator externus usually arises before the obturator nerve enters the obturator foramen. The nerve generally divides into two branches, one of which enters the superior border of the muscle, and the other passes to its external surface.

Some of the superior fasciculi of the obturator externus muscle may be separated from the main belly by the obturator nerve or its deep branch.

Adductor magnus. Plate II, Fig. 3; Plate VIII, Figs. 1 and 2. This muscle is developed from two distinct anlages, to one of which a branch from the obturator nerve is given, and to the other a branch from the sciatic nerve. These anlages are distinct in an embryo of 14 mm., but in one of 20 mm. (Plate VIII, Fig. 3) they have fused and a rearrangement of tissue has begun so that the three divisions of the muscle described by Poirier[7] are beginning to be distinct. The exact steps in this rearrangement of tissues I have been unable clearly to follow in the material at my disposal. In the adult muscle the obturator nerve usually gives off one or more branches which enter the main body of the superior division of the muscle, the adductor minimus, on its obturator surface about midway between the tendons of origin and insertion, and several branches which pass in between the larger fasciculi of the middle and inferior divisions of the muscle about midway between their tendons of origin and insertion. The branch from the sciatic nerve likewise enters between the main muscle bundles on the posterior surface of the middle and inferior divisions of the muscle about midway between their tendons of origin and insertion and usually sends a recurrent branch into the lower border of the superior division of the muscle. Not infrequently the nerve to the quadratus femoris muscle sends a branch into the upper margin of the superior division of the adductor magnus. In one instance a special branch of the sciatic was given to this portion of the muscle.

There is nothing to indicate that either the obturator or the sciatic branch is confined in its distribution to the tissue of the muscle mass to which it is originally sent. On the contrary it is exceedingly probable that the sciatic branch helps to innervate a portion of the obturator muscle mass and the obturator branch a portion of the sciatic muscle mass.

Comparative Anatomy and Variation in the Adductor Group.

In this group are included the obturator externus and the three adductor muscles. The pectineus also belongs anatomically and physiologically and probably in part also morphologically with this group (see p. 303). The

[7] Traité d'Anatomie, Tome 2, p. 229.

adductor magnus in most mammals is innervated merely by the obturator nerve and there is a special præsemimembranosus muscle (W. Leche) which extends usually from the tuber ischii to the medial side of the distal end of the femur parallel with the semimembranosus. In the gorilla, orang, and gibbon the præsemimembranosus is combined with the adductor magnus, as it is in man (W. Leche). In many forms the præsemimembranosus is more or less fused with the semimembranosus. It may be looked upon as derived phylogenetically from the semimembranosus or medial flexor of the thigh (A. Bühler, 03). In echidna the adductor magnus is innervated both by the obturator and sciatic nerves (W. Leche).

In urodeles the elements of the adductor group are probably contained in the pubi-ischio-femoralis externus and possibly also in part in the pubi-ischio-femoralis internus. The pubi-tibialis may represent the mammalian præsemimembranosus and the sciatic portion of the adductor magnus in man. In reptiles the adductor elements are contained in the pubi-ischio-femoralis externus (and the ischio-femoralis, Gadow). In the different groups of mammals there is considerable variation in the number of individual muscles into which the adductor musculature is divisible; from one to six according to Le Double (97). In man the chief variations noted have to do with the greater or less fusion of the different muscles into which the group is divided. The adductor magnus may be united by fasciculi or fused not only with the neighboring long adductor but also, owing to the origin of its posterior portion from the hamstring group, with the semimembranosus muscle. The adductor minimus portion of the adductor magnus is frequently fused with the quadratus femoris and may be supplied by the same nerve although this portion of the muscle belongs normally chiefly to the territory of the obturator nerve. The short adductor is frequently fused with the obturator externus.

b. Nerve Variation in the Adult.

1. Variation in the Origin of the Obturator Nerve.

In the great majority of instances the obturator, like the femoral, nerve arises chiefly from the 22d, 23d, and 24th spinal (2d, 3d, and 4th lumbar) nerves. Table XVI indicates the spinal roots from which the nerve arose in 246 instances and the various types of lumbo-sacral plexus with which the various modes of origin were associated.

2. Relations of the Nerves Springing from the Obturator Nerve to the Spinal Nerves.

In the case of the obturator nerve it is even more difficult than in case of the femoral nerve to trace with accuracy the relations of the nerves of distribution to the nerve roots. Examination of several nerves leads me to the belief that the bulk of the nerve fibres distributed to each of the nerves of distribution occupy in the obturator nerve as it approaches

TABLE XVI.

Type of Plexus from which the N. Obturatorius arises.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Frequency of Origin of the N. Obturatorius from: Nn. Sp. [XX][8] XXI XXII XXIII XXIV	Nn. Sp. [XX] (XXI) XXII XXIII XXIV	Nn. Sp. [XXI] (XXII) XXIII XXIV	Nn. Sp. (XXII) XXIII XXIV	Nn. Sp. [XXI] (XXII) XXIII XXIV XXV	Nn. Sp. (XXII) XXIII XXIV XXV	Total Number.
	A	XXIV	XXVI		1					1
Ant.	B	XXIV	XXVII		15	5	5			25
	C	XXIV chiefly to sacral plexus	XXVIII	2	42	10	9			63
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII	25		53	26			104
	E	XXIV-XXV, or XXV	XXVIII					6	12	18
Post.	F	XXIV	XXIX					6	9	15
	G	XXIV-XXV, or XXV	XXIX					4	16	20
Total Number.....				27	58	68	40	16	37	246

[8] The nerves enclosed in brackets contribute fibres to the N. cut. fem. lat., but probably not to other branches of the N. femoralis; those enclosed in parentheses contribute few fibres to the N. femoralis.

the obturator foramen a position approximately shown in the following diagram. The position occupied by the chief bulk of the fibres of each of the main nerves of distribution is shown in outline while the approximate area of the 23d spinal (3d lumbar) nerve is indicated by stippling, in this diagram.

3. Variation in the Branches of Distribution Arising from the Obturator Nerve.

In the great majority of instances the obturator nerve divides at the proximal border of the adductor brevis muscle in such a way that the nerves of supply to the gracilis muscle and to the adductor longus and brevis muscles pass in the anterior division of the nerve external to the

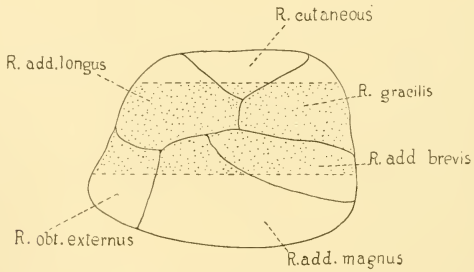


Fig. 5. Diagram to illustrate the position occupied in the obturator nerve by the nerve fibers going to the muscular and cutaneous branches.

adductor brevis muscles, while the nerves to the adductor magnus muscle and to the obturator externus muscles arise from the posterior division of the nerve. Very often the nerve to the obturator externus muscle is given off before the obturator nerve passes through the obturator foramen or above the place of division of the obturator nerve into anterior and posterior divisions. The nerves of distribution, the fibres of which occupy contiguous areas in the cross section of the main trunk shown above, may be associated for some distance in a common trunk before ultimately becoming independent.

In the following table are indicated the relations of the chief muscular and cutaneous branches of the obturator nerve in 88 instances. In all instances the branch to the obturator externus is included with the internal division of the obturator nerve, although in the majority of instances this branch was given off from the main trunk of the nerve before it split into anterior and posterior divisions. The articular and arterial

branches are not included in the table because the data concerning them are too incomplete. It is possible that in several instances the small cutaneous twig which anastomoses with the medial cutaneous nerve was lost in dissection. This table therefore indicates a minimum number of instances in which the obturator furnished a cutaneous branch. The same is true of the nerve to the pectineus muscle.

The parentheses in the table indicate the simultaneous division of a nerve trunk into the branches included within them. The commas indicate that the division of the nerves thus separated took place later than that of the combined nerve from the parent trunk.

TABLE XVII.

B—Nerve to adductor brevis muscle.
C—Cutaneous branch.
E—Nerve to obturator externus muscle.
G—Nerve to gracilis muscle.
L—Nerve to adductor longus muscle.
M—Nerve to adductor magnus muscle.

a. Branch to adductor brevis from anterior division.

No. of inst.	Anterior division.	Posterior division.
26	(E) (M)	(B) (L) (G)[9]
9	(E) (M)	(B) (L) (G, C)
	In all instances extensive distribution of cutaneous branch; in one half way down back of leg (481), in another nearly to ankle (693).	
12	(E) (M)	(B) (L, C) (G)[10]
	In five instances extensive distribution of cutaneous branch.	
18	(E) (M)	(B) (G, L)[11]
6	(E) (M)	(B) (G, L, C)
	In three instances the distribution of cutaneous branch was fairly extensive.	
5	(E) (M)	(B, L) (G)[10]
2	(E) (M)	(B, L, C) (G)
1	(E) (M)	(B, L) (C) (G)
1	(E) (M)	(B, G) (L)
1	(E) (M)	(B, G) (L, C)

[9] In one instance a branch to adductor minimus from nerve to quadratus femoris.

[10] In one instance a branch to the pectineus muscle was found.

[11] In two instances a branch to the pectineus muscle.

b. Branches to adductor brevis from anterior and posterior divisions.

2 (E) (B) (M) (B) (L) (G)

c. Branch to adductor brevis from posterior division.

4 (E) (B) (M) (G) (L)

1 (E) (B) (M) (G) (L, C)

III. THE SCIATIC NERVE.

In early embryonic life the separation between the tibial and peroneal nerves is well marked nearly to their origin from the sacral plexus. Near the plexus there intervenes between them a considerable amount of dense tissue (Plate II, Fig. 3) and more distally they are separated by the anlage of the fibula (Plates III, IV, and V).

a. Embryonic Development.

The *peroneal nerve* in an embryo 11 mm. long (Plate III, Fig. 2) may be traced as far as the middle of the dorsal side of the limb-bud. Four fairly distinct muscle anlages are visible along its course. The first of these, the gluteus medius mass, represents the anlage of the gluteus medius and minimus, the piriformis and the tensor fasciæ latæ, and toward it special branches are proceeding from the plexus. The second muscle mass represents the anlage of the gluteus maximus and the third that of the short head of the biceps. These two anlages adjoin one another. The fourth represents the anlage of the extensors of the ankle and peroneal muscles. Some differentiation is apparent between the anlages of the last two groups of muscles. In an embryo of 14 mm. (Plate IV, Fig. 2; Plate VIII, Fig. 4; Plate IX, Fig. 1) muscle differentiation has taken place in each of the anlages mentioned above and the anlage of the short extensor of the toes has appeared. To each muscle rudiment a nerve branch is given. In an embryo of 20 mm. (Plate V, Fig. 2; Plate VIII, Fig. 5; Plate IX, Fig. 2) muscle differentiation is more marked and the branches to each muscle resemble somewhat those of the adult.

The *tibial nerve* in an embryo of 11 mm. (Plate III, Fig. 1) extends to the middle of the plantar side of the leg. Along its course several muscle anlages may be seen. Of these the first is that of the obturator internus, the second that of the quadratus femoris, the third that of the hamstring muscles, the fourth that of the gastrocnemius—soleus group, and the fifth that of the deep muscles of the back of the leg. In an embryo of 14 mm. (Plate IV, Fig. 1; Plate VIII, Fig. 1; Plate IX, Figs. 3 and 4) individual muscles have appeared in each of the anlages mentioned and a muscle mass has appeared in the foot. In the leg the

lateral and medial plantar nerves are separated from one another. Nerves are given to the various muscle anlages. In an embryo 20 mm. long (Plate V, Fig. 1; Plate VIII, Figs. 2 and 3; Plate IX, Figs. 5 and 6) the muscles of the foot are beginning to be differentiated, the medial and lateral plantar nerves on the back of the leg have become fused and the branches to the various muscles somewhat resemble those of the adult.

b. Adult Conditions..

1. Separate Origin of the Peroneal and Tibial Nerves.

During early embryonic development, as mentioned above, the peroneal and tibial nerves arise separately from the plexus. In about 10% of instances studied at the Johns Hopkins University this condition was found present in the adult, the two nerves being separated by a portion of the piriformis muscle or more rarely by the whole muscle (see Bardeen and Elting, 01). Eisler, 92, found the condition in 18.1% of 123 plexuses and Paterson, 94, in 13% of 23 plexuses. The nerves arise separately from "normal," proximal or distal types of plexuses with about equal frequency.

2. Frequency of Variation in Origin of the Peroneal and Tibial Nerves.

In Tables XVIII and XIX are shown the frequency of various modes in origin of the tibial and peroneal nerves from the sacral plexus, and the types of plexus with which these various modes of origin were associated. No detailed explanation of these tables seems requisite. Tabulation of the relation of the various types of origin in relation to race, sex, and side of the body has revealed no facts of special interest, and hence tables covering these points are here omitted.

3. Relations of the Branches Springing from the Peroneal and Tibial Nerves to the Nerve Roots.

It is not often possible to trace back with certainty to their origin from the plexus the various branches springing from the peroneal and tibial nerves. It can be done only under special conditions and cannot be well carried out by students in the dissecting room. For this reason no attempt has been made to collect statistical data on this subject. The following diagram based on special dissections, indicates roughly the regions occupied by the chief nerve branches in the peroneal and tibial trunks, and their relations to the spinal roots of these nerves. Although the peroneal and tibial nerves are usually bound up on the back of the

TABLE XVIII.

Type of Plexus from which N. Peroneus arises.				Frequency of Origin of N. Peroneus from:						
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. [XXIII][12] XXIV XXV XXVI	Nn. Sp. XXIV XXV XXVI	Nn. Sp. XXIV XXV XXVI (XXVII)	Nn. Sp. XXV XXVI XXVII	Nn. Sp. (XXIV) XXV XXVI XXVII XXVIII	Nn. Sp. XXV XXVI XXVII (XXVIII)	Total Number.
	A	XXIV	XXVI		1					1
Ant.	B	XXIV	XXVII	1	6	7				14
	C	XXIV chiefly to sacral plexus	XXVIII	1	12	18				31
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII		5	26				31
	E	XXIV-XXV, or XXV	XXVIII			3	5		1	9
Post.	F	XXIV	XXIX			8		2		10
	G	XXIV-XXV, or XXV	XXIX			3	3	1	3	10
Total Number				2	24	65	8	3	4	106

[12] Fibres from 23d spinal nerve possible but not certain.

TABLE XIX.

Type of Plexus from which the N. Tibialis arises.				Frequency of Origin of the N. Tibialis from:								
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. - XXIV XXV XXVI	Nn. Sp. [XXIII][13] XXIV XXV XXVI (XXVII)	Nn. Sp. XXIV XXV XXVI (XXVII)	Nn. Sp. (XXIII)[13] XXIV XXV XXVI XXVII (XXVIII)	Nn. Sp. XXIV XXV XXVI XXVII (XXVIII)	Nn. Sp. XXV XXVI XXVII (XXVIII)	Nn. Sp. XXIV XXV XXVI XXVII XXVIII XXIX	Nn. Sp. XXV XXVI XXVII XXVIII (XXIX)	Total Number.
	A	XXIV	XXVI	1								1
Ant.	B	XXIV	XXVII		1	24						25
	C	XXIV chiefly to sacral plexus	XXVIII				5	58				63
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII					104				104
	E	XXIV-XXV, or XXV	XXVIII					7	11			18
Post.	F	XXIV	XXIX							15		15
	G	XXIV-XXV, or XXV	XXIX							8	12	20
Total Number.....				1	1	24	5	169	11	23	12	246

[13] Fibres from 23d spinal nerve possible but not certain.

thigh into a common trunk there seems normally to be no crossing of nerve fibres from one nerve to the other. Branches arising from each nerve may be bound for a certain distance into a common trunk provided that they occupy contiguous positions in the parent nerve, as indicated in the diagram Fig. 6.

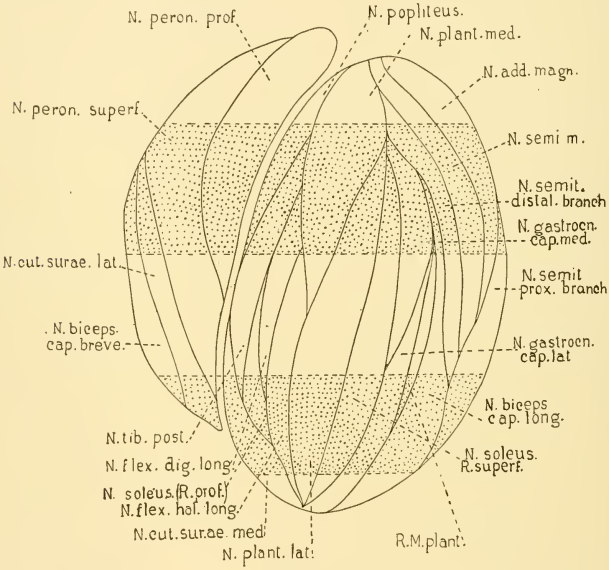


Fig. 6.
See text above.

IV. SUPERIOR GLUTEAL GROUP.

a. Embryonic Development.

1. General Features.

This group consists of the gluteus medius and minimus muscles, the piriformis, and the tensor fasciæ latæ. The last becomes distinct from the general muscle mass at a very early stage, the others are closely bound together during the earlier stages of differentiation in the anlage. Gräfenberg, **04**, has described the development of these muscles in man. In an embryo of the fifth week he describes a cone-shaped mass of dense

tissue the point of which extends toward the upper end of the femur. This he considers the anlage of the gluteus maximus, the piriformis, the gluteus medius and minimus, the tensor fasciæ latæ, quadratus femoris, and obturator internus. In the embryos of this period which I have studied I have not found an intimate union between the anlages of the gluteus medius group, the gluteus maximus, the obturator internus and the quadratus femoris groups. When differentiation of the muscles in this region begins the four anlages, though none of them sharply outlined, seem to me fairly distinct from one another as I have attempted to show in Plate III, Figs. 1 and 2.

In an embryo of 14 mm. (Plate VIII, Fig. 4) the m. tensor fasciæ latæ is quite distinct from the rest of the group. Gräfenberg states that at first it is closely connected with the anlage of the gluteus minimus. There is no connection between the anlages of the tensor fasciæ latæ and that of the gluteus maximus. The separation of the gluteus medius from the gluteus minimus is marked best in the region through which the superior branch of the superior gluteal nerve passes out to end in the tensor fasciæ latæ (Plate II, Fig. 3). The piriformis is still closely bound to the anlage of the two gluteals. I can find no connection between it and the gluteus maximus such as that described by Gräfenberg. The anlages of the two gluteal muscles and the piriformis pass distally into the proximal part of the back of the femur in the region where later the great trochanter will be developed. The gluteal anlages, closely applied to the anlage of the acetabulum, extend to the femoral margin of the embryonic ilium. The piriformis extends over the peroneal nerve toward but does not reach the pelvis. It is to be presumed that in those instances in which the peroneal nerve passes through the piriformis the course of development of the muscle toward the sacrum takes place on each side of the nerve. The dense tissue between the peroneal and tibial nerves in this region may represent an interneural process of this kind. It is continuous with the piriformis anlage.

Two distinct branches of the superior gluteal nerve may be seen. One of these extends to the tensor fasciæ latæ, the other ends in the anlage of the gluteus medius. The nerve to the piriformis is likewise beginning to grow toward this muscle.

In an embryo of 20 mm. (Plate VIII, Fig. 5) the great trochanter is becoming well marked, Bardeen, 05, and the attachments of the two deeper gluteal muscles and the piriformis begin to resemble those of the adult. The gluteal muscles have extended a considerable distance over the ilium the ala of which is much better developed than in the 14 mm.

embryo. The two muscles are clearly differentiated from one another, but the gluteus medius is partly fused to the piriformis and in this embryo the piriformis has not extended to the sacrum. In other embryos of about this stage the piriformis has, however, become attached to the sacrum. The distribution of nerves to these muscles is very similar to that found in the adult. The tensor fasciæ latæ extends distally over the thigh into the anlage of the tractus ilio-tibialis which at this period is but slightly marked.

In subsequent development the iliac ala increases in size and the muscles extend over it to their adult attachments. With the development of the anterior superior spine of the ilium the iliac attachment of the tensor fasciæ latæ is carried far from its original position near the back of the head of the femur.

2. Individual Muscles.

Tensor fasciæ latæ.—This is differentiated near the lateral edge of the anlage of the gluteus medius and minimus. According to Gräfenberg, 04, it is at first closely fused with this anlage and extends from the "Beckenschaufel" to the anlage of the great trochanter. In the specimens which I have studied the anlage of the muscle when it first becomes distinct has no skeletal attachment but lies near the gluteal anlage (Plate VIII, Fig. 4). From here it shifts laterally and its proximal extremity soon becomes attached to the ilium somewhat distal to the crest and behind the anlage of the anterior superior iliac spine. Distally it extends toward the lateral side of the knee (Plate VIII, Fig. 5) and is continued into the tractus iliotibialis which toward the end of the second month begins to be distinct.

In the adult the nerve usually enters the muscle about midway between its origin and insertion. This area corresponds to that first differentiated in the embryo.

It seems probable that that portion of the m. ilio-tibialis of urodeles and reptiles innervated by the sciatic nerve (the m. gluteo-rectus) represents the tensor fasciæ latæ of mammals. In different mammals the tensor fasciæ latæ varies greatly in development. It is said not to be present in monotremes and marsupials (W. Leche). It is large in all anthropoids except the orang (Le Double).

Gluteus medius and minimus.—These two muscles are differentiated in close association with one another and remain closely associated in the adult. The myoblastema from which they are derived lies close to the back of the embryonic skeleton near the junction of the femur with the pelvis (Plate VIII, Fig. 4). The anlage of the two muscles seems from

the first to extend distally into the anlage of the great trochanter, but proximally it extends only to the acetabular portion of the ilium. From here the muscles extend over the lateral surface of the iliac ala and finally reach the iliac attachments characteristic of the adult. The ascending branch of the superior gluteal nerve takes a course at first distal to the transverse branch, but as the gluteus medius grows toward the iliac crest the ascending branch is carried proximally across the transverse.

The gluteus medius and minimus muscles correspond with the ilio-femoralis of urodeles and reptiles, a muscle supplied by branches from both the femoral and sciatic nerves. In the monotremes the "gluteus medius" is a thin muscle which arises from the sacro-caudal vertebræ and is supplied by a branch of the peroneal nerve while the gluteus minimus and scansorius are represented by a mass of muscle which arises from the fascia lumbo-dorsalis, the lumbar and sacral vertebræ and the ilium and is innervated by branches of both the femoral and peroneal nerves. In all higher forms the gluteus medius-minimus musculature is innervated by branches which arise directly or indirectly from the peroneal portion of the sacral plexus (Westling, cited by Leche). It seems not unlikely that in the urodeles, reptiles and monotremes elements of the ilio-psoas musculature of higher forms are included in the ilio-femoral musculature. The more superficial and posterior part of the sciatic portion of the ilio-femoral anlage has given rise in the higher mammals to the gluteus medius and piriformis, the deeper and more anterior portion to the gluteus minimus and scansorius. The degree of separation of these various elements varies greatly in different mammals.

The variations of the two muscles which have been found in man are chiefly those of a greater differentiation than usual of individual muscles from the common anlage (*i. e.*, M. scansorius) or a partial or complete fusion of the muscles with one another or with the piriformis.

The piriformis.—This is differentiated from tissue at first closely associated with the gluteus medius and minimus (Plate VIII, Fig. 4). According to Gräfenberg the muscle anlage can from its first appearance be traced to the sacrum. While it is true that a dense mass of cells surrounding the sciatic nerve and its roots of origin can be followed back to the sacrum this condensed tissue is not, I believe, to be looked upon as the anlage of the piriformis, although the two are not at first sharply to be distinguished. Differentiation of the muscle is first clearly marked in the region between the sacral plexus and the anlage of the great trochanter. From here the developing muscle may be followed in older embryos toward its sacral attachment. In embryo XXII (Plate VIII, Fig. 5) the sacral attachment has not yet been reached. The region in which the nerve enters the adult muscle corresponds with the area in which muscle differentiation is first seen. As pointed out above, the differentiation of the muscle at a period preceding the fusion of the

TABLE XX.

Type of Plexus from which the N. Gluteus Superior arises:				Frequency of Origin of N. Gluteus Superior from:						
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. XXIV XXV	Nn. Sp. XXV	Nn. Sp. XXIV XXV XXVI	Nn. Sp. XXV XXVI	Nn. Sp. XXIV XXV XXVI (XXVII)	Nn. Sp. XXV XXVI (XXVII)	Total Number.
	A	XXIV	XXVI	1						1
Ant.	B	XXIV	XXVII	9		14				23
	C	XXIV chiefly to sacral plexus	XXVIII	16		44				60
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII	23		63		4		90
	E	XXIV–XXV, or XXV	XXVIII	2	1	4	7	2	2	18
Post.	F	XXIV	XXIX	2		8		1		11
	G	XXIV–XXV, or XXV	XXIX			6	5	1	3	15
Total Number.....				53	1	139	12	8	5	218

TABLE XXI.

Type of Plexus from which the Nerve to the Piriformis arises:				Frequency of Origin of Nerve to Piriformis from:					
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. XXIV XXV	Nn. Sp. XXV XXVI	Nn. Sp. (XXV) XXVI	Nn. Sp. XXVI XXVII	Nn. Sp. (XXVI) XXVII	Total Number.
	A	XXIV	XXVI			1			1
Ant.	B	XXIV	XXVII		4	3	2		19
	C	XXIV chiefly to sacral plexus	XXVIII	1	6	26	4	2	39
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII		8	23	5	8	44
	E	XXIV–XXV, or XXV	XXVIII		3	2	1	3	9
Post.	F	XXIV	XXIX		1	1	1	2	5
	G	XXIX–XXV, or XXV	XXIX			1		6	9
Total Number					22	67	15	21	126

peroneal and tibial nerves into a common trunk may account for the variation in the relations of those nerves to the muscle in the adult.

Although Gegenbaur and others have considered the piriformis to be derived from the caudo-femoral muscle of urodeles and reptiles, both comparative anatomical and embryological studies speak against this view. The caudo-femoral muscle of these lower forms is represented in many of the mammals by a caudo-femoralis (W. Leche), which typically extends from the caudal vertebræ to the lateral side of the distal half of the femur and runs parallel with the præsemimembranosus. As in the reptiles and urodeles so here the muscle toward its femoral insertion lies in front of the sciatic nerve while the piriformis normally runs dorsal to this nerve.

The piriformis is to be looked upon as an especially differentiated portion of the ilio-femoral muscle of urodeles and reptiles. In a considerable number of mammals it is not differentiated (some ungulates, etc.).[14]

In man the piriformis is frequently fused with the gluteus medius. Its origin may take place from the great sciatic notch instead of from the sacrum.

b. Nerve Variation in the Adult.

1. Variation in the Relations of the Superior Gluteal Nerve and of the Nerve of the Piriformis to the Nerve Roots.

The preceding tables, XX, XXI, indicate the frequency of certain modes of origin from the sacral plexus of the superior gluteal nerve and the nerve to the piriformis muscle and the relation of these modes of origin to certain types of lumbo-sacral plexuses. While there is some correspondence between an anterior or a posterior form of plexus and a "high" or "low" mode of origin of the nerves this correspondence is by no means perfect.

2. Variation in the Branches of Distribution.

Superior gluteal nerve.—Most frequently this nerve arises by two roots, one from the lumbo-sacral cord (4th-5th lumbar) and the other from the first sacral nerve. The trunk usually soon divides into two branches. The ascending branch is distributed mainly to the more dorsal part of the gluteus medius muscle in the middle third between its tendons. According to some authors it also sends fibres to the gluteus minimus muscle. I have found it much more frequently confined in distribution to the gluteus medius muscle. The transverse branch passes across the external surface of the gluteus minimus muscle about midway between its tendons and near the lateral border of the muscle passes beneath a

[14] According to Kohlbrugge, 97, the piriformis has a double origin, on the one side from the gluteal musculature, on the other from the metameric caudal muscles.

special slip of the muscle and terminates in the proximal portion of the middle third of the m. tensor fasciæ latæ. It gives branches of innervation to the gluteus minimus muscle, to the lateral portion of the gluteus medius muscle and to the tensor fasciæ latæ. The fibres of the ascending branch always arise from the plexus lower down than those of the transverse branch. Sometimes it arises as a separate branch from the first sacral nerve. It may then pass through the substance of the piriformis muscle and be associated with the nerve to the piriformis muscle.

Nerve to the piriformis muscle.—Very commonly the nerve to this muscle may arise from a loop connecting the first and second sacral nerves, but more often the branches arise directly from the first or second sacral nerve and pass into the substance of the muscle in the middle third between the tendons. The ascending branch of the superior gluteal nerve may send a ramus to the piriformis muscle. I have never seen a branch from the third sacral nerve to the piriformis such as those described by Weber, Hildebrandt, Valentine and Henle.

V. THE GLUTEUS MAXIMUS AND THE SHORT HEAD OF THE BICEPS.

The studies in comparative anatomy of Ranke, 97, Klaatsch, 02, and others have gone to prove the close morphological association of the gluteus maximus and the short head of the biceps.

It seems probable that both the short head of the biceps and the gluteus maximus are represented in the urodeles by the ilio-(femoro)-fibularis and in reptiles by the ilio-fibularis muscle which is supplied by the peroneal portion of the sciatic. In the mammals the proximal attachment of this musculature has extended well into the caudal region from the ilium. In the monotremes it is represented by a muscle which extends from the caudal region to the foot and in Echidna lies posterior to and does not cover the other glutei (Westling). In most of the higher forms it is divisible into three muscles, the superficial gluteus, or gluteus maximus, the femoro-coccygeus (Leche), and the gluteo-crural (Klaatsch). The superficial gluteus is inserted into the femur or into the fascia of the thigh. The femoro-coccygeus is inserted into the shaft of the femur, and the gluteo-crural into the fascia of the leg or into the fibula. The superficial gluteus and the femoro-coccygeus are not infrequently fused to form the gluteus maximus. The gluteo-crural is absent in some forms. In most mammals it extends as the tenuissimus from the caudal vertebræ or the gluteal fascia to the leg. In man and a few of the higher primates it arises from the femur and becomes applied to the tendon of the long head of the biceps to form the short head of this muscle. Klaatsch, 02, has given an especially valuable account of the gluteo-crural muscle. See also Windle and Parsons, 00.

In man the gluteus is not infrequently found divided into two portions, a condition normal in the embryo. The short head of the biceps not infre-

quently has a tendon of insertion more or less distinct from that of the long head. Its tendon of origin may be attached to the tuber ischii, the fascia covering the gluteus maximus, or the sacrotuberosal ligament.

a. Embryonic Development of Gluteus Maximus.

The gluteus maximus arises from an anlage which lies dorso-lateral to the anlage of the great trochanter (Plate III, Fig. 2). Its proximal edge overlaps and lies near but does not seem to be fused with the gluteus medius anlage. Distally it is slightly fused with the anlage of the short head of the biceps. Into the gluteus maximus anlage two nerves extend from the back of the sacral plexus.

In an embryo of 14 mm. (Plate II, Fig. 3; Plate VIII, Fig. 4) the gluteus maximus is quite distinct from the neighboring muscles.[15] It is beginning to show a division into two portions each of which is supplied by a separate nerve. The more distal of the two portions is continuous with the blastema of the femur. Proximally the muscle is extending over the gluteus medius and obturator internus anlages toward the ilium and sacrum. I find no primitive intrapelvic extension of the gluteus maximus such as that described by Gräfenberg, but the fascial extension which he describes from the dorsal muscles over the gluteal muscles is quite evident (see Plate II, Fig. 3).

In an embryo 20 mm. long (Plate VIII, Fig. 5) the gluteus maximus has extended from the trochanteric region where it first appears to the ilium, sacrum and coccyx. It is at this period very distinctly separated into two portions the more distal of which is inserted into the femur distal to the great trochanter while the more proximal is inserted into the fascia over the attachment of the distal portion. In the adult the two portions are only rarely thus distinct. The distal portion represents the femoro-coccygeus muscle so common in the lower mammals. In the younger embryos two nerves pass from the plexus to the muscle. In this embryo a special nerve is given to each portion of the muscle, but the two nerves arise by a common trunk from the plexus. The nerve to the superficial portion of the muscle curves toward the ilium and passes upwards on the deep surface of the muscle along a line about midway between the origin and insertion of the muscle. The nerve to the distal portion passes distally and enters its proximal margin (Plate VIII, Fig. 5).

[15] The early union with the piriformis described by Gräfenberg I have not found in any of the embryos I have examined, although I find, as he describes, an early transitory union between the anlages of the short head of the biceps and the gluteus maximus.

b. Variations in the Inferior Gluteal Nerve.

This nerve arises in the main from the first sacral nerve, but in part usually also from the lumbo-sacral cord; often from the 2d sacral, and rarely from the 3d sacral. Its roots may be superficially bound up with the trunks of origin of the posterior cutaneous nerve and not infrequently with the main sciatic trunk. In the great majority of instances the main trunk of the nerve divides into an ascending and a descending branch.

TABLE XXII.

Type of Plexus from which the N. Gluteus Inf. arises.				Frequency of Origin of N. Gluteus Inf. from:			
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. (XXIV) XXV XXVI	Nn. Sp. [XXIV] XXV XXVI (XXVII)	Nn. Sp. [XXV] XXVI XXVII (XXVIII)	Total Number.
	A	XXIV	XXVI	1			1
Ant.	B	XXIV	XXVII	12	6		18
	C	XXIV chiefly to sacral plexus	XXVIII	24	27	3	54
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII	29	40	5	74
	E	XXIV-XXV, or XXV	XXVIII		9	1	10
Post.	F	XXIV	XXIX	1	6	3	10
	G	XXIV-XXV, or XXV	XXIX		10	1	11
Total Number.....				67	98	13	178

The ascending branch curves upwards on the under surface of the gluteus maximus muscle midway between the tendons of origin and insertion. The descending branch is distributed in the middle third of the deep distal portion of the muscle. The fibres of the descending branch have a more distal origin than those of the ascending branch. In the adult the two branches often arise separately from the plexus. The table above shows the frequency of origin of the nerve from various groups of spinal nerves and the frequency with which each is associated with a given type of plexus.

c. Embryonic Development of the Short Head of the Biceps.

In an embryo of 11 mm. (Plate 3, Fig. 2) the anlage of the short head of the biceps extends along the distal half of the fibular margin of the femur dorso-lateral to the peroneal nerve. Proximally it is continued to the anlage of the gluteus maximus.

In an embryo of 14 mm. (Plate II, Fig. 3; Plate VIII, Fig. 4) it does not extend proximally quite to the femoral insertion of the gluteus maximus. Distally it is beginning to be attached to the tendon of the long head of the biceps. The nerve to the muscle which at the former stage was not evident may at this stage be seen entering the fibular margin of the muscle.

G. D. Thane mentions an instance in which the nerve to the short head of the biceps arose in connection with the inferior gluteal nerve from the sacral plexus.[16]

In an embryo 20 mm. long (Plate VIII, Fig. 5) both the femoral and distal attachments of the muscle are well marked.

VI. THE MM. OBTURATOR INTERNUS, GEMELLI AND QUADRATUS FEMORIS.

These constitute a distinct group of muscles which are differentiated on the ischial side of the anlage of the hip joint. Although closely associated, the anlage of the obturator internus and gemelli seems to be from its earlier stages of differentiation distinct from that of the quadratus femoris. I do not find the anlages of these muscles fused at an early stage with the gluteal anlages as described by Gräfenberg, 04. When they first appear (Plate III, Fig. 1) the anlage of the quadratus femoris has a somewhat more anterior position than that of the obturator internus. This may account for its nerve supply in the adult from a more proximal set of spinal nerves.

a. Embryonic Development.

Obturator internus and gemelli.—An indistinct region of tissue differentiation near the ischium in Embryo CIX, length 11 mm. (Plate III, Fig. 1) I take to be the anlage of the obturator internus and the gemelli. To it a nerve is given from the sacral plexus. The anlage of these muscles is much more distinct in an embryo 14 mm. long (Plate VIII, Figs. 1 and 4). Here it may be seen extending from the anlage of the great

[16] Quain's Anatomy, 10th ed.

trochanter across and then upwards for a short distance on the pelvic surface of the ischium toward the obturator foramen. No distinction can at this time be made between the obturator internus and the two gemelli. From the sacral plexus a nerve branch may be seen extending across the outer surface of the muscle. Beneath the muscle another nerve may be traced to the anlage of the quadratus femoris.

In an embryo 20 mm. long (Plate VIII, Fig. 5) the obturator internus has extended well over the obturator foramen and in its growth into the pelvis has carried its nerve in the same direction. The gemelli cannot yet be clearly distinguished from the obturator internus. A good description of the architecture of these muscles in the adult and of the distribution of nerves to them is given in the *Traité d'anatomie humain* of Poirier and Charpy. In the adult the chief variations in structure are those of a greater or less independence of the gemelli and a greater or less extent of the pelvic attachments of the obturator internus.

Quadratus femoris.—This is differentiated comparatively early in a region lying between the anlage of the great trochanter and that of the tuber ischii (Plate III, Fig. 1; Plate VIII, Fig. 4). It soon forms attachments which correspond well with those of the adult muscle (Plate VIII, Fig. 5). In the embryo, as in the adult, the nerve enters the deep surface of the muscle near the junction of the middle and ischial thirds. In the adult the muscle is frequently fused either with the inferior gemellus or with the adductor minimus. Its nerve of supply may extend into the adductor minimus.

The quadratus femoris, gemelli, and obturator internus muscles of mammals are apparently related to the ischio-femoral musculature of urodeles and the pubi-ischio-femoralis posterior (Gadow) of reptiles. Among the mammals the obturator internus is said not to be found in the monotremes (W. Leche) but it occurs in most, although not all, of the higher forms. The degree of isolation of the gemelli and the mode of attachment of the obturator internus vary considerably in different mammals. The quadratus femoris seems to be a fairly constant muscle in the mammalian series. In a considerable number of mammals, however, it is innervated by the obturator nerve instead of by a special branch from the sacral plexus (see W. Leche). I do not know of an instance of this kind being reported as a variation in man. The innervation of the adductor minimus portion of the adductor magnus by the nerve to the quadratus femoris is, however, frequent and rarely this nerve may send a branch to the M. obturator externus. The adductor minimus is normally supplied chiefly by the obturator nerve. In Talpa the quadratus femoris and obturator externus are fused and the combined muscle is supplied both from the obturator nerve and from the sacral plexus (W. Leche).

TABLE XXIII.

Type of Plexus from which the Nerves arise.				Frequency of Origin of the Nerve to the M. Obturator Internus from:				Frequency of Origin of the Nerve to the M. Quadratus Femoris from:				
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. XXIV XXV XXVI	Nn. Sp. XXV XXVI XXVII	Nn. Sp. [XXV] XXVI XXVII XXVIII	Total Number.	Nn. Sp. XXIV XXV	Nn. Sp. XXV	Nn. Sp. XXIV XXV XXVI	Nn. Sp. XXV XXVI	Total Number.
	A	XXIV	XXVI									
Ant.	B	XXIV	XXVII	1	6		7	5		2		7
	C	XXIV chiefly to sacral plexus	XXVIII		18		18	9		9		18
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII		11	1	12	4		8		12
	E	XXIV-XXV, or XXV	XXVIII		1	1	2		1		1	2
Post.	F	XXIV	XXIX		2	1	3			3		3
	G	XXIV-XXV, or XXV	XXIX		2	4	6				6	6
Total Number				1	40	7	48	18	1	22	7	48

b. Nerve Variation.

1. Variation in the Origin of the Nerves to the Obturator Internus and Quadratus Femoris Muscles.

In Table XXIII the frequency of variation in the origin of the nerves to these muscles is shown. The nerve to the quadratus femoris muscle arises usually from the lumbo-sacral cord and the 1st sacral nerve (24th, 25th, and 26th spinal nerves.) Not infrequently the 25th spinal nerve is the most distal nerve to furnish fibres to this nerve. This condition occurs usually in the more proximal forms of plexus. In the more distal forms of plexus the 25th and 26th spinal nerves furnish the fibres for this muscle.

TABLE XXIV.

Association of the Branches Distributed to the Obturator Internus, Gemelli, and Quadratus Femoris Muscles.	Number of Instances Associated with Plexus Type:[17]						Total Number.
	B	C	D	E	F	G	
Branch 1, to Mm. obturator int. and gemellus sup. Branch 2, to Mm. quadratus femoris and gemellus inf.[18]	1	5	4	2	1		13
Branch 1, to M. obturator int. Branch 2, to M. gemellus sup. Branch 3, to Mm. quadratus femoris and gemellus inf.		1					1
Branch 1, to M. obturator int. Branch 2, to Mm. quadratus femoris and both gemelli.						3	3
Branch 1, to Mm. obturator int. and gemellus sup. Branch 2, to M. quadratus fem., gemellus inf., and adductor magnus.		2					2
Total Number.	1	8	4	2	1	3	19

[17] For types of plexus see preceding table.

[18] In one instance a branch was traced to the M. obturator externus.

The nerve to the obturator internus muscle arises usually from the (24th) 25th, 26th, and 27th spinal nerves. Rarely the 26th spinal nerve is the most distal nerve to furnish fibres to it and occasionally in distal forms of plexus the 28th spinal nerve may do so.

It is difficult to trace these nerves back to their roots of origin. The charts on which these tables are based are those recording the most accurate dissections of these nerves. They are, however, of positive rather than negative value and it is possible that a more extensive origin than here indicated was present in some of the plexuses here recorded.

2. Variation in the Nerves of Distribution.

The frequency of this variation is indicated in Table XXIV. Only those charts are used for tabulation which were based on the more accurate dissections of the distribution of the nerves to the muscles. Most frequently the nerve to the obturator internus muscle furnishes a branch to the superior gemellus muscle while that to the quadratus femoris muscle furnishes a branch to the inferior gemellus muscle. Occasionally a separate branch passes from the sacral plexus to the superior gemellus muscle. In distal forms of plexuses the nerve to the quadratus femoris muscle may furnish branches to both gemelli muscles. Not infrequently the branch to the quadratus femoris muscle is continued into the proximal portion of the adductor magnus muscle. This condition has been described by Wilson, 89. In one instance I have followed a branch to the M. obturator externus.

VII. THE HAMSTRING MUSCLES.

a. Embryonic Development.

1. General Features.

In an embryo 11 mm. long (Plate III, Fig. 1) two branches from the tibial portion of the sciatic nerve represent nerves to the hamstring muscles. They terminate in a mass of tissue on the plantar side of the femur. The more proximal of the two nerves represents the proximal branches to the long head of the biceps and the semitendinosus; the more distal nerve, that to the distal part of the semitendinosus and the long head of the biceps and to the semimembranosus and adductor magnus muscles.

In an embryo 14 mm. long (Plate VIII, Fig. 1) the various muscles mentioned are distinctly differentiated. But a single nerve branch is given to the sciatic portion of the adductor magnus (at this period a distinct muscle not closely fused with the obturator portion) and to the semimembranosus. To the semitendinosus and to the long head of the biceps proximal and distal branches are given. About the terminus of each motor nerve the muscle differentiation is best marked. The tendinous attachments at each extremity of the muscles are indefinite. Proximally they fuse with the ischial blastema.

In an embryo 20 mm. long (Plate VIII, Fig. 3) the muscles of this group are attached by tendons to the skeleton. The obturator and sciatic portions of the adductor magnus have become fused.

2. Individual Muscles.

Adductor magnus.—See p. 313. That portion of this muscle which is attached to the distal end of the femur represents the praesemimembranosus of the lower mammals and belongs primitively to the hamstring group.

Semimembranosus.—This muscle arises from a special anlage in close association with that of the sciatic portion of the adductor magnus (Plate II, Fig. 3; Plate VIII, Fig. 1). The belly of the muscle becomes distinct before the tendons. In an embryo of 20 mm. (Plate VIII, Fig. 3) there is a flat tendon of origin which is closely applied to the adductor magnus and which arises from the ischium. The tendon of insertion fuses with the tibial blastema near the back of the knee joint. The nerve enters near the center of the muscle belly. In the adult the nerve enters by several branches into the substance of the muscle about midway between the tendinous attachments of the muscle bundles composing it. The superior branches curve upwards either on the surface or within the substance of the muscle. There is much individual variation in the exact mode of distribution of the branches of the nerve to this muscle.

The semimembranosus is probably represented in urodeles by a part of the (caudali)-pubi-ischio-tibialis and in reptiles by a portion of the flexor tibialis internus. In most mammals it arises from the ischium or pubis, runs parallel with, and may be incompletely differentiated from the præsemimembranosus, mentioned above in connection with the adductor magnus, and is inserted into the tibia. It may be fused with the semitendinosus. A. Forster, 03, has shown that although in the lower mammals the semimembranosus is a flexor and may send a tendinous expansion to the plantar aponeurosis, in apes and monkeys it is chiefly an internal rotator of the leg. In many mammals it is associated with a caudo-femoral (W. Leche) muscle which extends from the caudal vertebræ to the distal end of the femur.

In man it may be longitudinally doubled, may be partially fused with the adductor magnus or the semitendinosus and may arise from the ischial spine or the sacro-tuberosal ligament as well as from the tuber ischii.

Semitendinosus.—This muscle is formed from two anlages, one of which is differentiated in close conjunction with the anlage of the ischial tuberosity, the other more distally. These anlages correspond with the two parts of the muscle found in the adult and to each a separate nerve is given (Plate VIII, Fig. 1). The anlages are visible in an embryo of 14 mm. and the muscle is well differentiated in one of 20 mm. (Plate VIII, Fig. 3). In the latter the tendinous inscription which subdivides the muscle is as distinctly marked as in later life. The tendon of insertion is inserted relatively more distally in the 20 mm. embryo than in the adult.

In the embryo as in the adult a special nerve is given to each portion of the muscle. The nerve to the more proximal part arises from a more distal set of spinal nerves than that to the more distal part. It gives rise to branches which enter between the bundles of the proximal portion of the muscle about midway between the tendon of origin and transverse tendinous inscription. The more distal nerve enters the distal portion of the muscle by branches which have a similar distribution with respect to that portion.

The semitendinosus is probably represented in urodeles in the (caudali)-pubi-ischio-tibialis and in reptiles by a portion of the flexor tibialis internus. In monotremes it arises with the semimembranosus from the tuber ischii, is inserted into the tibia, and is supplied both by the obturator and sciatic nerves. In the higher forms it is either single as in man, double as in several insectivores, or has two heads of origin, one of which usually springs from the tuber ischii, the other from the caudal vertebræ. This last, according to W. Leche, is probably the most primitive condition. The tendinous inscription of the semitendinosus marks the region where the two heads join the common belly in this type of muscle. Humphrey believed the tendinous inscription to mark the place where in the lower vertebrates the caudo-crural joins somewhat perpendicularly the flexor musculature of the thigh. In most of the lower mammals and in all the apes the tendon of insertion sends fibrous expansions far down in the crural fascia and together with similar expansions from the biceps and gracilis helps to form a sheath for the tendon of Achilles (Parsons, 04).

In man the semitendinosus and long head of the biceps sometimes arise independently from the ischium, a variation which is supposed by Le Double, 97, to be a reversion to a primitive condition in which the two muscles were quite independent. Klaatsch, 02, on the other hand, states that in the lowest mammals the muscles are more closely united than in the higher. In the human embryo the two muscles are closely united from their earliest differentiation and the union extends relatively more distal than in the adult. The semitendinosus may be more or less fused in the adult with the semimembranosus or connected by fasciculi with the long head of the biceps.

The semitendinosus in the embryo extends more distally in the crus than is normal in the adult. The fascial extension of the tendon in the adult is, however, frequently well marked and may be muscular (Gruber, 86). Proximally the semitendinosus in man may be reinforced by fasciculi from the pelvis or coccyx. These fasciculi may join the muscle at its tendinous inscription (Le Double). In the normal development I have found nothing that seems to represent a "latent" caudal head of the muscle. It is noteworthy that the proximal segment of the semitendinosus is innervated by a more distal set of spinal nerves than the distal segment (see above). The proximal end of the biceps is likewise innervated by a more distal set of spinal nerves than the distal end of that muscle. The proximal ends of these two muscles may therefore represent a caudo-femoral anlage shifted distally into the thigh.

Biceps, caput longum.—The long head of the biceps is differentiated from a special anlage which, near the ischial tuberosity, is closely fused with that of the semitendinosus. This anlage is well marked in an embryo of 14 mm. (Plate VIII, Fig. 1) and the muscle is differentiated in one of 20 mm. (Plate VIII, Fig. 3). To the anlage in the 14 mm. embryo two nerves are given each of which is associated at its origin with corresponding nerves to the semitendinosus. In the 20 mm. embryo two nerves are likewise given to the muscle, but in this instance the nerves arise nearly in conjunction with one another from the tibial portion of the sciatic nerve.

In the adult two nerves are commonly distributed to the muscle. One of these enters the proximal portion of the muscle, the other in the distal third. The terminal branches of these nerves are distributed across the muscle bundles of the biceps about midway between their tendons of origin and insertion, but nearer the proximal than the distal tendon. The more distal nerve sends back recurrent branches across the muscle bundles when the more proximal nerve is absent or ill developed.

Biceps, caput breve. See p. 332.

The long head of the biceps or lateral crural flexor is probably represented in the urodeles by the ischio-flexorius and in reptiles by the flexor tibialis externus. In the mammals it usually arises from the tuber ischii and is inserted into the tibia or into the fascia of the leg, often as far as the foot. In marsupials it arises from the tuber ischii and the caudal vertebræ. In the carnivora and some of the other mammals it has occasionally a double origin. As in the case of the semitendinosus the caudal origin of this muscle is looked upon, however, by many investigators as a caudo-femoral muscle inserted into the lateral flexor rather than as a true head of the muscle. According to Testut the long head of the biceps represents a muscle which primitively arose from the ilium and the coccyx. The sacrotuberosal ligament represents a transformation of that portion of the muscle which originally extended between the ilio-sacro-caudal region and the present origin of the muscle from the tuber so that the ligament may be looked upon as the tendon of insertion of the muscle. In the human embryo the ligament develops after the anlages of the ischial tuberosity and the long head of the biceps have appeared. It apparently is differentiated from the tuber ischii toward the ilium, sacrum, and coccyx. In the human adult fasciculi from the coccyx, sacrum, or sacrotuberosal ligament to the head of the biceps are frequent.

The distal insertion of the muscle in most of the lower mammals takes place further down the leg than in man. In most of the lower mammals, according to Parsons, 04, as mentioned above, extensions from the tendons of the semitendinosus, gracilis, and biceps into the crural fascia serve to form a sheath for the tendon of Achilles. According to A. Forster, 03, in fœtuses the insertion of the biceps takes place into the sural fascia and even

TABLE XXV.

Order in which the Branches to the Hamstring Muscles arise from the Tibial Division of the Sciatic Nerve.

1st Branch.	No. Inst.	2d Branch.	No. Inst.	3d Branch.	No. Inst.	4th Branch.	No. Inst.	5th Branch.	No. Inst.	6th Branch.	No. Inst.
Ta (1)	12	Ta (2) + Ba, b	1	A m + m + Tb	1				1		
		Ta (2)	1	Tb	1	Ba, b	1	M	2	A m	1
		Ba	4	Bb	2	A m + M	2	Tb	1		
				Tb	1	A m + M	1	Bb			
				Bb + Tb	1	A m + M	1				
		Ba, b	6	A m + M + Tb	4						
				A m + M	1	Tb	1				
				Tb	1	A m + M	1				
Ta + Ba	1	Bb	1	Tb	1	A m + M	1				

TABLE XXV.—*Continued.*

1st Branch.	No. Inst.	2d Branch.	No. Inst.	3d Branch.	No. Inst.	4th Branch.	No. Inst.	5th Branch.	No. Inst.	6th Branch.	No. Inst.
Ta + Ba, b	8	A m + M + Tb	4								
		A m + M	4	Tb	4						
Ba, b	8	Ta	8	A m + M + Tb	3						
				Tb	3	A m + M	3				
				A m + M	2	Tb	2				
Ba	3	Ta	3	Bb	2	A m + M	1	Tb	1		
						Tb	1	A m + M	1		
				A m + M	1	Bb	1				
Ta + Ba, b + Tb	1	A m + M	1								
Ta, b	1	Ba, b	1	A m + M	1						

Ta = branch to proximal segment of M. semitendinosus.
Tb = branch to distal segment of M. semitendinosus.
Ba = proximal branch to long head of M. biceps.
Bb = distal branch to long head of M. biceps.
Am = branch to M. adductor magnus.
M = branch to M. semimembranosus.

in young children the attachment to the head of the fibula is weak. In embryos of the third month the tendon of insertion of the biceps can be followed for some distance down the fibular side of the leg but there seems to be some attachment to the fibula.

b. Nerve Supply.

1. Relations of the Nerves of the Muscles of the Hamstring Group to the Spinal Nerves.

In the adult it is difficult to trace back with certainty to the spinal nerves the nerves distributed to these muscles. In general the special dissections which I have made have revealed conditions which correspond well with the data given by G. D. Thane in Quain's Anatomy, Vol. III, Part II, p. 331, which in turn are based on data derived from Paterson and Eisler. According to the description there given the nerve to the adductor magnus arises from the 4th and 5th lumbar nerves, that of the semimembranosus from the 4th and 5th lumbar and 1st sacral nerves. The two nerves of the semitendinosus arise from the 5th lumbar and 1st and 2d sacral nerves. I have found the inferior nerve arising from the 4th and 5th lumbar and 1st sacral, the superior from the 1st and 2d sacral nerves. The nerves for the long head of the biceps arise from the 1st, 2d, and 3d sacral nerves; that of the short head of the biceps from the 5th lumbar and 1st, or 1st and 2d, sacral nerves. In Text Fig. 6 the relation of these nerves to the sciatic nerve is diagrammatically shown. A study of Plate III, Fig. 3, and Plate VIII, Figs. 1 and 3, will show that a distribution of spinal root fibres corresponding with this scheme would follow from the more direct paths to the muscle anlages open to fibres growing out from the sacral spinal nerves when the muscle anlages first appear.

2. Relation of the Nerves of the Hamstring Muscles to the Sciatic Nerve.

To the semitendinosus and to the long head of the biceps, as a rule, two separate nerves are given, one going to the proximal the other to the middle or distal third of each muscle. Occasionally each of these nerves may be doubled and not infrequently the nerves to each muscle are combined for a part of their course in a common trunk. For the semimembranosus and adductor magnus as a rule a single branch springs from the sciatic nerve. This branch soon divides into separate branches for each muscle. The various nerves mentioned spring at varying heights from the sciatic nerve and are variously combined in the branches which spring from this nerve. In Table XXV the relative origins of the

branches of the sciatic nerve are tabulated and there is shown the frequency with which the different combinations occurred in 34 plexuses. From this table it may be seen that the nerve to the proximal segment of the semitendinosus muscle is most frequently the first of these branches to arise. Very frequently this branch is associated with one or both of the branches distributed to the long head of the biceps. Often the latter branches are the first to arise from the sciatic nerve. Rarely the branch to the distal segment of the semitendinosus arises in common with that to the proximal segment. When the most proximal branch given off is that to the proximal segment of the semitendinosus the next branch is usually that to the long head of the biceps. The more distal of the branches to the long head of the biceps may arise low down from the sciatic nerve. The nerve to the distal segment of the semitendinosus arises about on the level and often in common with the nerve to the adductor magnus and semimembranosus.

VIII. PERONEAL MUSCLES.

a. Embryonic Development.

During the sixth week the anlage of the peroneal muscles becomes separated from that of the long extensors of the toes and the tibialis anterior (Plate IX, Fig. 1). Between the two anlages runs the n. peroneus superficialis. The anlage of each peroneal muscle begins at the same time to become distinct. Schomburg, **00**, has described a connection in early embryonic development between the peroneus brevis and the extensor digitorum brevis. In the embryos of corresponding stages which I have examined the two muscles are distinctly separated as shown in Plate IX, Fig. 1.

The *m. peroneus longus* occupies the more proximal and superficial position. It lies dorso-lateral to the upper end of the fibula. Its proximal extremity is some distance from the tibia. The distal extremity is continued into a tendon which can be followed to the neighborhood of the base of the fifth metatarsal where it is lost in tissue not yet distinctly differentiated.

In somewhat older embryos the tendon of the muscle may be followed as it develops across the sole of the foot toward the base of the first metatarsal. In an embryo 20 mm. long the tendon is intimately fused with the scleroblastema of the foot and can be distinctly followed only partially across the sole. In an embryo 30 mm. long the tendon can be followed to the first metatarsal, but it is considerably later than this when the tendon becomes free in its sheath. In an embryo 14 mm. long,

Plate IX, Fig. 1, the tendon passes lateral to the anlage of the lateral malleolus. In one of 20 mm. it passes behind this anlage (Plate IX, Fig. 2).

Proximally in an embryo 20 mm. long (Plate IX, Fig. 2) the origin of the muscle extends to the lateral condyle of the tibia next to the attachment of the m. flexor digitorum longus. At this period the two heads characteristic of the adult muscle may be distinguished.

A nerve may be seen entering the anlage of the peroneus longus in embryo CXLIV (Plate IX, Fig. 1) and in embryo XXII (Plate IX, Fig. 2) two nerves to the muscle may be seen. One of these enters the deep surface of the anterior head, the other passes distally into the posterior head.

From the peroneal nerve as it passes beneath the muscle two branches usually arise in the adult. One of these passes to the central third of the anterior portion of the muscle, the other extends down across the middle third of the deeper muscle bundles which run obliquely from the fibula to the tendon of the muscle. The latter branch may arise from the n. peroneus superficialis and it may extend to supply the m. peroneus brevis.

The *M. peroneus brevis,* (Plate IX, Figs. 1 and 2) arises proximally under cover of the peroneus longus and relatively higher up on the fibula than in the adult. It lies a little more on the flexor side of the leg than the peroneus longus. When first developed the tendon of insertion of the muscle is closely associated distally with that of the m. peroneus longus. It lies somewhat near the m. extensor digitorum brevis, but, as mentioned above, I can find no such intimate union with this muscle as that which Schomburg describes as lasting till the third month of development. It is attached to the base of the fifth matacarpal in an embryo 20 mm. long (Plate IX, Fig. 2).

In an embryo 14 mm. long (Plate IX, Fig. 1) a branch of the peroneal may be seen entering the muscle. In one 20 mm. long (Plate IX, Fig. 2) this branch may be readily followed beneath the peroneus longus to the peroneus brevis which here occupies a more distal position than in the 14 mm. embryo. In the adult the nerve may arise either from the distal nerve to the peroneus longus or from the n. peroneus superficialis. The nerve enters the proximal margin of the muscle and extends distally about midway between the origin and insertion of the constituent fibre bundles.

In amphibians the femoro-fibularis, which extends from the lateral epicondyle of the femur to the fibula probably represents the peroneal musculature of the higher vertebrates. In the reptiles two peroneal muscles are recog-

nized, the peroneus anterior and peroneus posterior (Gadow, 82). The m. peroneus anterior extends in most forms from the proximal extremity of the fibula to the base of the fifth metatarsal. The peroneus posterior in crocodiles is more or less fused with the gastrocnemius and extends from the extensor musculature of the thigh to the calcaneus. In Hatteria and many saurians it is more or less fused with the peroneus anterior and extends from the lateral condyle of the femur to the outer side of the fifth metatarsal (Gadow, 82). In the mammals the peroneal group consists in most forms of three muscles, a peroneus longus, peroneus brevis, and peroneus extensorius.

The peroneus longus may be inserted into the base of the fifth metatarsal or into various structures in the sole of the foot, as far as the base of the first metatarsal. In Ornithorhynchus the tendon of this muscle may be followed to this last insertion.

The peroneus brevis and peroneus extensorius in Ornithorhynchus constitute a muscle, one part of which sends tendons to the extensor surface of the terminal phalanges of the first four toes, the other to that of the fifth toe. In marsupials the peroneus brevis is distinct from the peroneus extensorius. The latter arises from the lateral condyle of the femur and from the fibula and sends tendons to the second and fifth toes. In rodents the peroneus extensorius sends tendons to the fourth and fifth toes. In carnivora it sends a tendon to the fifth toe. In some apes the peroneus extensorius is differentiated and sends a tendon to the fifth toe. In others it is not isolated from the peroneus brevis. In man a peroneus extensorius (peroneus quartus of Le Double) is not infrequently found as a variation under most diverse forms. Most frequently the tendon only is isolated and is inserted into the fifth metatarsal, cuboid, calcaneus, etc. The tendon of the peroneus brevis frequently sends expansions to the tendon of the fifth toe, that of the fourth toe, the metatarsal of the fourth toe, etc. In normal embryonic development, however, the peroneal musculature does not seem to become connected with the extensor tendon plate.

b. Nerve Distribution.

The nerves to the peroneal muscles probably arise from the more distal spinal nerves which go to form the peroneal nerve, but this cannot be satisfactorily determined by dissection.

The nerves to the peroneal muscles (brevis and longus) may arise from the main trunk of the n. peroneus, from the n. peroneus superficialis, or from both. In 15 out of 20 instances a single branch passed from the n. peroneus superficialis to the peroneus brevis, in one instance two such nerves were given, in four instances the nerve arose from the more distal branch to the peroneus longus.

In three instances out of 20 a single nerve branch ran from the n. peroneus to the peroneus longus muscle; in 8 instances two such branches. In four of these cases the second branch sent a nerve of supply to the

peroneus brevis. In four instances the nerves of supply of the peroneus longus arose from the n. peroneus superficialis (by one branch in one instance). In five instances a proximal branch (in one instance, two) arose from the n. peroneus and a more distal branch from the n. peroneus superficialis.

In some of the instances above cited the nerves of supply subdivided before entering the muscle.

IX. MUSCULATURE OF THE EXTENSOR SIDE OF THE CRUS AND FOOT.

a. Embryonic Development.

1. General Features.

In an embryo of 11 mm. (Plate III, Fig. 2) the peroneal nerve extends over the dorsal surface of the limb-bud and ends in a mass of slightly differentiated myogenous tissue, the anlage of the extensor muscles of the leg and foot. This anlage is more or less fused with the anlage of the peroneal muscles.

In an embryo 14 mm. long (Plate IX, Fig. 1) the peroneal nerve has given rise to the nn. peroneus superficialis and profundus. The n. peroneus profundus may be traced to the region between the bases of the first two metatarsals. Above and on each side of it may be distinguished muscle anlages representing the extensor muscles of the leg and foot. To these muscle anlages nerves are given as shown in the figure. The tendons of the extensor digitorum and extensor hallucis proprius are represented by a sheet of tissue in which the segmentation is just beginning. The conditions here described correspond well with those pictured by Schomburg, oo, except in a few minor details to which attention is called in considering the development of the individual muscles.

In an embryo of 20 mm. (Plate IX, Fig. 2) the individual muscles and their tendons, as well as the nerves distributed to them, indicate clearly relations corresponding in many features with those characteristic of the adult.

2. Individual Muscles.

Tibialis anterior.—The anlage of this muscle becomes distinct from the general dorsal myogenous sheet of the limb-bud during the sixth week. In an embryo of 14 mm. (Plate IX, Fig. 1) the muscle anlage is most distinctly differentiated in the region where the two nerves are extending into it. From here it may be followed distally into a broad tendon which fades out over the region of the first cuneiform and the base of the first metatarsal. In an embryo of 20 mm. (Plate IX, Fig. 2)

the muscle has made tendinous attachments which correspond with those of the adult and the chief nerve branches have extended for a considerable distance into the muscle.

In the adult muscle as a rule several small branches extend into the upper extremity of the muscle and one or two large branches enter the middle third of the muscle. Within the muscle these branches run in intramuscular septa and are distributed chiefly across the middle third of the component muscle bundles. These run obliquely from their origin from the tibia and surrounding aponeurotic sheets to the tendon of insertion which arises high in the muscle.

The anterior tibial muscle is probably represented in the urodeles by the femoro-tibial muscle which extends from the lateral epicondyle of the femur to the tibia and os tarsale tibiale and is more or less fused with the femoro-digital or long extensor muscle. In reptiles and mammals the anterior tibial is fairly constant in general relations. It arises in most mammals from the proximal end of the tibia and is inserted into the lateral side of the tarsus or into the first metatarsal. In many mammals, including monkeys and apes, the muscle is partially divided into two portions from one of which a tendon goes to the metatarsal of the big toe (abductor hallucis longus), the other to the first cuneiform. This division may affect merely the tendon of insertion or extend into the belly of the muscle. In man there is not infrequently (25% of bodies, Le Double) a similar division of the terminal tendon but this rarely extends to the belly of the muscle. Schomburg, 00, describes a distinct division of the anlage of the tibialis anterior in the embryo into two parts, that toward the tibial side representing an abductor hallucis longus. This division does not appear in the embryos I have examined.

Extensor digitorum longus.—From the central portion of the dorsal myogenous sheet the extensor digitorum longus and the extensor hallucis longus are differentiated simultaneously (Plate IX, Fig. 1). The extensor digitorum occupies a position relatively more fibularwards than in the adult. It is broad and ends below in a broad flattened process, or tendon plate, at the center of the dorsum of the foot. There is no very distinct division into special tendons. Two nerve branches extend to the deep surface of the muscle where this overlies the n. peroneus profundus. Schomburg, 00, has described conditions in a six weeks embryo which do not differ very essentially from those here given.

In an embryo of 20 mm. (Plate IX, Fig. 2) we find that tendinous attachments have extended to the digits from the tendon plate and that proximally the muscle has extended more toward the tibia. The nerve supply corresponds with that of the adult.

In the adult as a rule two chief nerve branches arise from the n.

peroneus profundus. One of these runs to the muscle near its upper extremity and passes distally across the central third of the obliquely placed fibre bundles of the proximal portion of the muscle. The other branch leaves the n. peroneus profundus more distally, extends to the middle or lower third of the muscle and then distally across the middle third of the obliquely placed fibre bundles of the lower portion of the muscle and across the corresponding fibre bundles of the m. peroneus tertius. The two nerves may be bound up in one trunk or their place may be taken by a considerable number of branches, but in 9 cases out of 10 essentially the arrangement described may be found.

The extensor digitorum longus and extensor hallucis longus are represented in urodeles by the femoro-fibulæ-digiti I-V which extends from the lateral epicondyle of the femur and from the fibula to the foot and thence by means of tendinous processes to the phalanges (Hoffmann). In reptiles the two muscles are probably[20] also represented by the extensor digitorum longus which in most reptiles arises from the lateral epi-condyle of the femur and is inserted by tendinous slips into the bases of some of the metatarsals. In chelonians it is inserted by tendons into the phalanges (Gadow, 82). In the mammals the extensor digitorum and extensor hallucis are distinct in most forms. The extensor digitorum arises chiefly from the proximal part of the tibia and is united to the back of the toes by tendinous process which vary considerably in different forms (Ruge, 78). In man doubling of the digital tendons and aberrant tendon slips are very frequent (Le Double). Early in embryonic development, as we have seen above. the tendons are represented by a tendon plate. In the adult the tendons may be connected by an uninterrupted aponeurotic lamella or by tendinous slips, conditions normal in many of the lower mammals. Occasionally in man slips from the tendons of the long digital extensor pass to the first, fourth, or fifth metatarsals (Testut). This corresponds to the attachment of the extensor tendons to the metacarpals found in reptiles. In the human embryo the extensor tendon plate is at first connected with the metatarsal scleroblastema but is gradually separated from this as development proceeds.

M. peroneus tertius.—Schomburg, 00, finds this muscle distinct from the extensor digitorum pedis longus even in the sixth week. In the two embryos in which I have made the most careful study of these muscles (144, length 14 mm., Plate IX, Fig. 1; and 22, length 20 mm., Plate IX, Fig. 2) I have been unable to find a sharp distinction between the two muscles, although the tendon of the peroneus tertius in embryo 22 is quite distinct from that of the extensor digitorum longus. Schomburg finds the tendon of the m. peroneus tertius runs at first toward the third

[20] It is possible that the m. extensor hallucis proprius of reptiles is homologous with the extensor hallucis longus of mammals. It seems more likely that it should be classed with the dorsal pedal muscles.

metatarsal, but I have found no such condition in the embryos studied. The tendon when differentiated runs toward the fifth digit. The nerve supply of this muscle, described above in connection with the extensor digitorum longus, serves to support the contention of Gegenbaur that the m. peroneus tertius is but a differentiated portion of the extensor digitorum pedis longus. It varies greatly in size and is frequently fused with the m. extensor digitorum longus. Its tendon may terminate on the fourth metatarsal. Rarely a tendon slip is given to the extensor tendon of the little toe (Le Double).

M. extensor hallucis longus. Even at an early stage this muscle may be distinguished from that of the m. extensor digitorum longus as Schomburg, 00, has pointed out. Its tendon at first is fused with the tendon plate of the extensor digitorum longus (Plate IX, Fig. 1), but soon begins to acquire some independence (Plate IX, Fig. 2).

The nerve of supply in embryos CXLIV and XXII enters the muscle near the center of its oblique tibial border. As a rule in the adult the nerve approaches the tibial border and passes distally across the oblique muscle bundles midway between their origin and insertion. This single trunk may, however, be replaced by two or more branches arising independently from the n. peroneus profundus.

Variations in the muscle are most frequently found with respect to its tendon of insertion. The tendon may divide into two or more parts. In one instance it has been found sending a slip to the second toe. The body of the muscle may be more or less fused with that of the m. extensor digitorum longus.

The extensor hallucis longus is to be looked upon as an especially differentiated deep portion of the extensor digitorum longus. Occasionally in man there is found arising from the fibula a special long extensor of the second toe (Gruber, 75). This is homologous with the extensor indicis proprius of the forearm. Chudzinski, 74, has described a deep extensor sending tendons to the first metatarsal, to the second and third, and to the fourth and fifth toes, an arrangement corresponding somewhat to one normal in several mammals (marmot, porcupine, beaver, Le Double). Both the extensor hallucis longus and the extensor digitorum longus are connected with the dorsal tendon plate in the embryo at an early stage. Normally a tendon for the first toe develops from the deep surface of this plate in connection with the extensor hallucis longus muscle, but the variations found in the adult show that the primitive tendon plate may be variously subdivided during embryonic development. The tendon of the extensor hallucis may send a tendon to the first metatarsal or to the second toe, etc. (W. Gruber, 75).

Mm. extensor digitorum brevis.—This muscle becomes differentiated beneath the extensor tendon plate and is best developed on the fibular side

of the dorsum of the foot. At the end of the sixth week, as pointed out by Schomburg, the muscle mass is not differentiated into special parts (Plate IX, Fig. 1), but toward the end of the second month the bellies of which it is composed and their tendons begin to stand out distinctly (Plate IX, Fig. 2). The differentiation of the terminal tendons begins on the fibular side and extends toward the tibial. The nerve to this muscle mass arises at an early stage from the n. peroneus profundus and extends across its deep surface. In the adult this nerve extends across the component muscle bundles about midway between their tendons of origin and insertion.

Extreme variability is shown in the form of this muscle in the adult. It may be absent or be reduced to two or three bundles or there may be an unusual development of the muscle and the differentiation into bellies corresponding to the digital tendons. The *m. ext. hallucis brevis* is the most frequently isolated of these bellies.

The extensor digitorum brevis doubtless represents the remains of an intrinsic dorsal pedal musculature relatively better developed in urodeles and reptiles than in most mammals. In most urodeles (Ribbing, 06) and reptiles (Gadow, 82) the extensor tendons of the toes arise from these pedal muscles and the "extensor digitorum longus" tendons are inserted into the bases of certain of the metatarsals. The great variation in the development of the extensor digitorum brevis in man is well known. It seems to be relatively better developed in the embryo than in the adult.

b. Nerve Distribution.

The relations of the nerves supplying the muscles under consideration to the spinal nerves cannot be clearly made out by dissection. It is probable, however, that the nerves supplied to the more tibially situated muscles contain the greater number of the fibres springing from the 4th lumbar nerve, and the nerves passing to muscles situated most to the fibular side contain the greater number of fibres from the 1st sacral nerve.

Variation in the Branches of Distribution Arising from the N. Peroneus Profundus.

The nerve to the extensor digitorum brevis seems to be constant in its general relations, although the height at which it springs from the main trunk varies greatly.

The nerves to the remaining muscles show considerable variation owing to the fact that the nerves to a given muscle may arise as successive branches from the main nerve trunk or they may be combined

into a single nerve of distribution which has a proximal origin and as it passes distally gives off successive branches which pass to the middle third of the obliquely placed fibre bundles comprising the muscle. Usually the nerve to the peroneus tertius arises in common with the nerve that is distributed to the more distal portion of the m. extensor digitorum longus. The following table, XXVI, shows the number of branches which passed in 20 instances from the main trunk to each of the muscles under consideration. Often a branch subdivides before entering the muscle. In the part treating of the individual muscles the most frequent form of nerve distribution for each muscle is described.

TABLE XXVI.

Tibialis anterior.

	No. of instances.
1 br. to proximal portion. 1 br. to center of muscle	7
2 brs. to proximal portion. 1 br. to center of muscle	6
1 br. to proximal portion. 2 brs. to center of muscle	3
2 brs. to proximal portion. 2 brs. to center of muscle	4
	20

The branches to the proximal portion are closely associated with an articular branch to the knee and in almost all instances arise from the peroneal nerve trunk before the n. peroneus profundus separates from the n. peroneus superficialis.

Extensor digitorum longus (e. d. l.) and peroneus tertius (p. t.).

1 br. to proximal portion e. d. l. 1 br. to central area e. d. l. and p. t.	8
1 br. to e. d. l.[21]	5
1 br. to proximal portion of the e. d. l. 1 br. to central area e. d. l. 1 br. to p. t.	5
1 br. to proximal portion e. d. l.	1
1 br. to proximal portion e. d. l. 1 br. to central area e. d. l., to extensor hallucis, and to p. t.	1
	20

Extensor hallucis.

1 branch	11
2 branches	4
3 branches	4
Branch arose with distal branch to ext. dig. longus	1
	20

[21] In one instance two separate branches to p. t.; in one, one branch to p. t.; in three instances p. t. not present.

X. MUSCULATURE OF THE PLANTAR SIDE OF THE CRUS AND FOOT.

a. General Features.

In an embryo 11 mm. long (Plate III, Fig. 1) the tibial nerve divides below the knee into two branches. Of these that on the tibial side represents the medial plantar, that on the fibular side the lateral plantar nerve.[22] The lateral plantar branch descends to the tarsus, the medial plantar nerve not so far distally. Near the knee a mass of slightly differentiated tissue lying superficial to the nerve represents the gastrocnemius-soleus group of muscles. Beneath the nerves beyond this region a mass of slightly differentiated tissue represents probably the anlage of the deep muscles of the calf and possibly of some of the musculature of the sole of the foot.

In an embryo 14 mm. long (Plate IX, Figs. 3 and 4) the muscles of the plantar side of the leg are so far differentiated that the individual muscles can be fairly clearly made out. In the drawing for the sake of definiteness the outlines of these muscles are made diagrammatically sharp but the main relations shown are true to the conditions found in the embryo. Two groups of muscles may be distinguished, a superficial lateral group composed of the gastrocnemius, soleus, and plantaris; and a deep more medially placed group consisting of the flexor hallucis longus, flexor digitorum longus, the popliteus, and the tibialis posterior. The gastrocnemius group is connected by a mass of tissue with the blastema of the calcaneus. The two long flexor muscles are connected by condensed tissue with a flat aponeurotic "foot-plate" from which tendinous processes extend to the blastema of the metatarsals and toes. The medial and lateral plantar nerves extend independently from the region of the knee to the foot. Near where they arise there is a plexiform arrangement of the fibres of the tibial nerve and from the back of this plexus arise the nerves to the deep muscles of the back of the leg and to the deep surface of the soleus muscle. The nerves to the gastrocnemius-soleus group, with the exception just mentioned, arise from the plantar surface of the tibial nerve proximal to where this changes its course from the thigh into the leg. In the foot the medial plantar nerve spreads out superficial to the pedal aponeurosis while the lateral plantar nerve crosses medially beneath it. Along the course of the medial plantar

[22] In the article by Bardeen and Lewis, 01, the two divisions of the tibial nerve are represented combined into a single trunk too far distally.

nerve in the foot a mass of slightly differentiated tissue represents probably the anlage of the musculature subsequently innervated by this nerve.

The general relations of the plantar nerves at this period are strikingly similar in many ways to the plantar nerves in the crus of the lower mammals as recently pictured by McMurrich in this journal (04) and offer analogies with types there pictured by him for the lower vertebrates. The chief difference between the nerves of the plantar side of the crus of mammals and that of the reptiles and amphibia is, as McMurrich has pointed out, the path for the fibers going to the medial side of the foot. In the mammals the nerve fibers take a course superficial to the deep muscles of the crus; in the inferior vertebrates they take a course in part beneath the deep muscles. In the amphibia and reptiles the nerve fibers for the medial side of the foot are more or less bound up with the nerves to the deepest muscles of the crus; in the mammals they are more or less bound up with nerves to the more superficial muscles. The nerve for the lateral side of the foot runs in most forms between the superficial and the deep musculature of the crus.

In an embryo 20 mm. long (Plate IX, Figs. 5 and 6) the various muscles of the plantar side of the leg are much more highly differentiated than in the 14 mm. embryo. The soleus and gastrocnemius muscles have begun to extend tibialwards over the tibial nerve. The tendon of Achilles is well differentiated. The long flexor muscles are attached to an aponeurosis from which tendons extend to the digits. The popliteus muscle is clearly marked. The tibialis posterior is inserted into the side of the skeleton of the foot near the base of the first digit. In the foot the anlages of most of the intrinsic muscles can be distinguished but here the muscles are but incompletely differentiated. A group of muscles innervated by the lateral plantar nerve is to be distinguished from one innervated by the medial plantar nerve.

The lateral and medial plantar nerves in this embryo are fused into a common trunk as far as the ankle. The nerves to the gastrocnemius-soleus group arise from the plantar surface of the tibial nerve in the thigh. To the deep surface of the soleus, however, a branch is given which arises from the deep surface of the tibial nerve in the leg. From this surface arise the nerves for the deep muscles of this region. In the foot the distribution of nerve branches to the muscles corresponds with that found in the adult.

b. Embryonic Development and Variation in the Nerve Supply in the Adult of Each of the Chief Groups of Muscles.

The development of the individual muscles of the back of the leg and foot can best be followed by taking them up according to the groups

which develop from common anlages. We shall therefore first take up the gastrocnemius-soleus group, then the deep musculature of the back of the leg, then the musculature innervated by the lateral plantar, and finally that innervated by the medial plantar nerve. The nerve supply of the muscles of the back of the crus is taken up after treating the embryonic development of the two sets of muscles in this region; the nerve supply of the plantar musculature is taken up after considering the differentiation of the plantar muscles.

1. Development of the Gastrocnemius-soleus Group.

M. gastrocnemius.—As pointed out by Schomburg, oo, the lateral portion of the flexor plate of the leg gives rise to the gastrocnemius and soleus muscles. The anlage of the gastrocnemius is the more lateral and superficial of the two muscles and shows two incompletely separated heads (Plate IX, Fig. 3). These heads are connected by a fairly dense tissue with the anlage of the calcaneus but do not extend across the tibial nerve to the femur. During the latter half of the second month the heads of the gastrocnemius develop rapidly. In an embryo of 20 mm. (Plate IX, Fig. 5) the lateral head of the gastrocnemius has formed a tendinous attachment above the lateral condyle of the femur while the medial head has not yet quite reached its final destination. The nerves to the gastrocnemius enter each head of the muscle soon after the anlages appear. The nerves may be readily distinguished in an embryo of 20 mm. (Plate IX, Fig. 5).

In the adult the fibre bundles of each head of the gastrocnemius take an oblique and nearly parallel, though somewhat diverging, course from the tendons of origin to the tendon of insertion. The nerve to each head enters about the middle third of the superior margin of the muscle and its main branches take a course distally across the obliquely running fibre bundles, a course corresponding to the course of the nerve in the embryo.

M. soleus.—The anlage of this muscle is closely associated with that of the gastrocnemius. It lies beneath and projects beyond the tibial margin of the gastrocnemius (Plate IX, Fig. 4). It arises on the upper end of the fibula and distally extends into an anlage of the tendon of Achilles. At first it is as large as the gastrocnemius. During subsequent development it extends over the posterior tibial nerve to be attached to the tibia. This attachment is not completed in an embryo of 20 mm. (Plate IX, Fig. 6). The nerves for the muscle arise at an early stage as shown in Plate IX, Fig. 4. Their distribution in an embryo of 20 mm. is shown in Plate IX, Fig. 6.

In the adult the superior nerve to the soleus enters the superficial surface near the superior border and divides into two main branches, one for the tibial and one for the fibular side. The inferior nerve to the soleus divides, usually before it enters the muscle, into two branches, one for the distal portion of the fibular, the other for the distal portion of the tibial side of the muscle. From both nerves branches may usually be followed both to the main body of the muscle and to the specialized bi-pennate portion visible on its deep surface.

M. plantaris.—According to Schomburg, 00, the anlage of this muscle arises proximal to the soleus and on the tibial side of the gastrocnemius. In embryo CXLIV, length 14 mm., the muscle mass is not clearly differentiated from the anlages of the soleus and gastrocnemius but what I

TABLE XXVII.

Amphibia.	Lacertilia.	Opossum.	Higher Mammals.
Plantaris sup. med. [Ischio-flexorius, Hoffmann.]	Plantaris sup. med. [Gastrocnemius, cap. int., Gadow.]	Gastrocnemius med.	Gastrocnemius med.
	Plantaris sup. lat. [Gastrocnemius, cap. ext., Gadow.]	Gastrocnemius lat.	Gastrocnemius lat.
Plantaris sup. lat. [Femoral head sup. flexor.]	Plantaris sup. access. [Flex. long. dig., cap. fem., Gadow.]	Plantaris.	Plantaris.
	Plantaris sup. tenuis. [Flex. long. dig., cap. access., Gadow.]		Popliteus. (Sup. portion)
Plantaris prof. III. [Flex. subl. dig., Hoffmann.]	Plantaris profundus III-II. [Flex. long. dig., cap. int., Gadow.]	Gastrocnemius lat. (Soleus portion.)	Soleus.
Plantaris prof. II. [Fem. fib. metatars. I-III. Hoffmann.]		Flexor fibularis.	Flexor fibularis.

take to be the anlage of the plantaris is a small mass of tissue situated anterior to the main soleus mass and partly covered by the gastrocnemius (Plate IX, Fig. 4). Even in embryo XXII, length 20 mm., the muscle cannot be made out distinctly. I have represented in Plate IX, Fig. 6, what I take here to be the anlage of the plantaris muscle. It is closely associated with the lateral head of the gastrocnemius. No traces of the tendon were found in the early embryos I have studied, nor did Schomburg find any in the leg reconstructed by him.

Comparative anatomy of the gastrocnemius-soleus group.—McMurrich in this journal has recently (04) given an important account of the comparative anatomy of the crural flexors from the standpoint of muscle layers as seen in cross-section. He tabulates the relationships of the gastrocnemius-soleus group as shown in Table XXVII.

In the development of the human embryo it has been shown that two fairly distinct chief myogenous regions are to be distinguished on the plantar side of the crus and that one of these gives rise to the gastrocnemius-soleus group, the other to the deeper muscles of the back of the leg. Both Eisler (1895) and McMurrich have performed a distinct service in again emphasizing that in the vertebrate series a superficial plantar musculature of the crus is to be distinguished from a deep plantar musculature. In many mammals, at least, including man, the two layers of musculature are separated by a fascial septum which passes from the fibular to the tibial side of the leg and in which run the main nerves and blood-vessels of the back of the crus. In the reptiles also there appears to be a similar fairly distinct division between the superficial and the deep plantar muscles of the crus. In them, however, tne muscles called plantaris superficialis tenuis and plantaris superficialis accessorius by McMurrich seem to be related proximally to the superficial muscula-

TABLE XXVIII.

Urodela.	Reptilia.	Marsupalia.	Man.
Ischio Flexorius.	Crural Tendon of flex. tib. ext.	Fascial Insertions of Flexors of Knee.	Fascial Insertion of Biceps, Semitendinosus and Gracilis.
Plantaris sup. minor, (Eisler.) [Plant. prof. III, minor, Mc.M.]	Plantaris sup. med.	Gastrocnemius med.	Gastrocnemius med.
Plantaris sup. major, (Eisler). [Plant. prof. III, Mc. M.]	Plantaris sup. tenuis.	Plantaris.	Plantaris.
Plant. sup. lat. (Mc. M.)	Plantaris sup. lat.	Gastrocnemius lat.	Gastrocnemius lat.
	Plantaris sup. access.		Soleus (all but deep portion.)

ture while distally they are inserted into the deep musculature. The plantaris superficialis tenuis lies chiefly superficial to, the plantaris superficialis accessorius, chiefly deeper than the nerve trunks which correspond with the nn. plantaris medialis and lateralis of the mammals (rr. superficiales medialis and lateralis of McMurrich). In the amphibia there seems to be a distinct division between the deeper musculature and a superficial group of muscles composed of the muscles called by McMurrich the plantaris superficialis medialis, the plantaris superficialis lateralis and the plantaris profundus III. Comparing the conditions found during embryonic development of the human crus with those present in the legs of the lower mammals and inferior vertebrates I should prefer to rearrange McMurrich's table as shown in Table XXVIII.

It seems probable that the muscles into which the superficial musculature of the plantar surface of the crus becomes divided are not perfectly homologous in the amphibia, reptiles, and mammals, although there are some obvious similarities.

In the mammals the homologies seem more certain. McMurrich considers the medial head of the gastrocnemius to be a muscle primitively distinct from the lateral head. He bases this conclusion on the fact that in many of the lower mammals each head forms a distinct muscle. The ontogeny of the muscle in man indicates that the two heads are derived from an anlage situated on the fibular side of the leg. The twisting of the tendon of Achilles may be explained by the shifting which the muscle undergoes during ontogeny. Embryological development in man supports the idea advocated by McMurrich that the plantaris is a derivative of the deeper portion of the lateral head of the gastrocnemius. When absent it is likely that this separation has failed to take place during ontogeny. In many mammals it is not differentiated (several edentates, carnivora, etc.); in others, especially in some rodents (Leche), it is highly developed. The soleus is considered by McMurrich to be derived from the profundus musculature of the crus. It seems to me likely that the deep portion of the soleus, innervated by the distal nerve to that muscle may be thus derived from the profundus musculature although I have been able to distinguish no such special anlage in the development of the muscle in man. In the monotremes the soleus is bound up with the lateral head of the gastrocnemius. This arises from the epiphysial process of the fibula. It forms a part of the lateral head of the gastrocnemius in marsupials, in most edentates, in the chiroptera and galeopithecidæ, several carnivora, ungulates, and prosimians (Leche). The great number of mammals in which it is thus undifferentiated as a distinct muscle indicates strongly that its phylogenetic as well as its ontogenetic origin is, in the main at least, from an anlage common to it and the gastrocnemius. Eisler, 95, regards it as derived from the gastrocnemius lateralis.

The variations in the muscles of the soleus-gastrocnemius group in man seem to be essentially due to a greater or less separation of the original anlage into independent muscles. The fascial extension of the biceps, semitendinosus and gracilis, which I take to represent the plantaris superficialis medialis (McMurrich) of the amphibian crus, may be muscular instead of tendinous and may be somewhat fused to the gastrocnemius.

2. Development of the Deep Muscles of the Back of the Crus.

a. M. Popliteus.—In an embryo 14 mm. long (Plate IX, Fig. 4) I have been unable to distinguish clearly a popliteus muscle. The anlage of the muscle doubtless lies in the dense tissue posterior to the tibial nerve and proximal to the anlage of the m. tibialis posterior. There is a differentiation of tissue there which indicates this. This tissue is outlined in the drawing. In an embryo of 20 mm. (Plate IX, Fig. 6) the muscle is well defined, has the skeletal relations characteristic of the adult and at its distal border there enters a well marked nerve of supply. Schomburg does not mention this muscle in his article.

In the adult the nerve usually enters the muscle near the center of its distal edge. Often some of the branches of this nerve extend into the

posterior surface of the muscle. Rarely a slender second branch enters the superior margin of the muscle (2 in 25 instances).

The place of the popliteus is taken in the lower mammals, the amphibia and reptiles by an interosseous muscle, pronator tibiæ, which passes obliquely from the fibula to the tibia. A popliteus muscle corresponding essentially to that of man is found in nearly all mammals except the monotremes and marsupials. A popliteus in addition to a pronator tibiæ is likewise described for Myrmecobius (Leche). The popliteus is said to be absent in most chiroptera (Leche). In the dog in addition to the popliteus there is a small fibulotibial muscle in the proximal part of the interosseous space. A similar muscle (the peroneo-tibialis, Gruber) has been found in a number of mammals and not infrequently as a variation in man (128 times out 860 instances, Gruber). It seems probable that the popliteus is an especially differentiated portion of the fibulo-tibial muscle of the lower vertebrates, and that its origin has extended from the fibula to the lateral condyle of the femur. Eisler, 95, considers it homologous with the brachialis anterior of the arm. According to McMurrich the muscle in the mouse receives two nerve branches, one associated with that for the soleus from the "internal popliteal stem," the other from the deep muscle nerve of the crus. The former is supplied to the more tibial oblique-fibered portion of the muscle, the latter to the more vertical fibular portion. From these facts McMurrich concludes that the popliteus is a compound muscle consisting of a portion derived from the "plantaris superficialis" and a portion which represents a part of the pronator tibiæ of the marsupials and the interosseous of the lower vertebrates. That it is therefore similar to the pronator teres of the arm. While this may be true of the muscle in some of the mammals it does not seem to be true for the muscle as it is found in man. A double innervation is infrequent in man. During embryonic development the muscle appears to come from a single anlage which lies deeper than the tibial nerve. Gordon Taylor and Victor Bonney, 05, conclude that the popliteus is homologous with the deep portion of the pronator teres while the superficial portion of the pronator teres is homologous with the gastrocnemius. Occasionally in man a second head may arise medially from above the lateral condyle. This may possibly be equivalent to the superficial portion of the pronator teres. According to Le Double the m. popliteus biceps coincides frequently with the absence of the plantaris.

b. Deep cruro-pedal group. M. flexor hallucis longus.—The anlage of this muscle is distinct from those of the other muscles of the calf in an embryo of 14 mm. (Plate IX, Figs. 3 and 4). Lateral to the anlage lies the calcaneus, the tendon of Achilles and the distal end of the soleus. On the tibial side it slightly overlaps the anlage of the tibialis posterior. Proximally it extends nearly to the head of the fibula. It lies beneath the n. plantaris lateralis which in this embryo separates high up from the n. plantaris medialis. Distally it terminates in an aponeurosis common

to it and the m. flexor digitorum longus. The nerve enters the proximal extremity of the muscle anlage.

In an embryo 20 mm. long (Plate IX, Figs. 5 and 6) the muscle occupies a relatively somewhat more proximal position and is somewhat more under cover of the soleus. It is attached to the blastema of the shaft of the fibula and distally is inserted into the deep surface of the plantar aponeurosis. The nerve runs along and enters the tibial margin of the muscle.

In the adult the nerve or nerves to the muscle run along its tibial margin or deep surface and send twigs into its substance.

M. flexor digitorum longus.—This is differentiated from an anlage medial to that of the m. flexor hallucis longus. In the 14 mm. embryo (Plate IX, Fig. 3) it lies beneath the n. plantaris medialis which gives two branches to the upper extremity of the anlage. The muscle extends into a somewhat irregular plantar aponeurosis of which mention has been made in connection with the m. flexor hallucis longus. The tendons are partially differentiated. The anlage of the muscle nearly covers that of the m. tibialis posterior. Schomburg found in the leg he reconstructed that the tibial side of the muscle had not reached the tibia. In embryo CXLIV this is also true. The tibial attachment has begun to take place in embryo XXII, length 20 mm. (Plate IX, Fig. 5). In this embryo also the pedal aponeurosis has become still further differentiated into tendons, but it is not yet possible to distinguish clearly the tendons belonging to the fibular flexor (flexor hallucis longus) from those belonging to the tibial flexor (flexor digitorum longus). Two nerves enter the muscle on its superficial surface. One of these extends to the fibular side of the muscle, the other to the tibial side. A similar arrangement is usually found in the adult.

M. tibialis posterior.—This muscle is formed from the deeper layer of the tibial portion of the flexor anlage near the lateral portion of the lower half of the tibia (Plate IX, Fig. 4). Its tendon is differentiated early and may be followed to the anlage of the navicular. In subsequent development, as pointed out by Schomburg, it develops in a proximal and lateral direction (Plate IX, Fig. 6). Its nerve enters near the tibial border of the anlage. In the adult the nerve enters the posterior surface of the muscle in its proximal third and gives off one or two branches for the tibial fasciculus. The main trunk descends across the centers of the fasciculi arising from the fibula.

Comparative anatomy of the deep plantar muscles of the crus.—Eisler, 95, and McMurrich, 04, consider that the flexor fibularis (hallucis) is derived from a layer primarily superficial to the layer from which the flexor

tibialis (digitorum) and tibialis posterior are derived. McMurrich[23] bases this idea chiefly on the supposition that the flexor fibularis is supplied by the equivalent of the ramus superficialis medialis, while the flexor tibialis is supplied from the ramus profundus. In man, at least, the nerves passing to the two muscles are very frequently bound up for some distance in a common trunk. The flexor tibialis and tibialis posterior seem, however, to be more intimately connected during ontogeny than is either of these muscles with the flexor fibularis. In many mammals the tibialis posterior is absent (Leche). In these it may be undifferentiated from the flexor tibialis. On the other hand in several mammals the tibialis posterior is doubled, the deeper portion sending a tendon to various structures in the tarsus, or even to the base of the first phalanx of the big toe (Le Double, 97). The intimate relations between the tibial and fibular flexors are revealed by the fasciculi which so frequently have been found passing from one to the other as well as by their tendons (see Le Double, 97).

The tibial and fibular flexors are inserted primarily into a deep plantar aponeurosis in which tendons are developed in accordance with varied functions of the foot and digits (Keith, 94). The arrangement of the tendons varies greatly in different forms. In many forms the flexor tibialis is rudimentary. In the chiroptera it is highly developed. For the variation of the tendons in the anthropoids and man see Le Double, 97.

3. Nerve Supply of the Muscles of the Back of the Crus in the Adult.

a. Relation of Muscle Branches to the Spinal Nerves.

The difficulty of tracing these nerves back to their sources from the spinal nerves is so great that no statistical study of the subject has been attempted. It is evident, however, that the main bulk of the nerve fibres distributed to the gastrocnemius-soleus group has in general a somewhat more distal origin than those going to the deep muscles of the calf. The special dissections which I have made serve in the main to support the spinal nerve origins given in Quain's Anatomy. These are as follows: popliteus, 4th and 5th lumbar, 1st sacral; soleus, 5th lumbar, 1st and 2d sacral; gastrocnemius, 1st and 2d sacral; deep musculature of the calf, 5th lumbar, 1st and 2d sacral. The nerve to the plantaris is given as arising from 4th and 5th lumbar and 1st sacral, but the 5th lumbar, 1st and 2d sacral nerves seem to be the more probable sources of supply.

[23] According to McMurrich the flexor tibialis and tibialis posterior of the mammals are represented in the reptiles (Lacertilia) and amphibia (urodeles) by the plantaris profundus I (tibialis posterior of Gadow). The flexor fibularis is according to this author derived from a portion of the plantaris profundis III-II of reptiles (flexor longus digitorum, caput internum, Gadow) and the plantaris profundus II of the urodeles (femoro-fibulæ-metatarsales I-III, Hoffmann).

b. Order of Origin from the Tibial Nerve.

The most proximal branches given off are those to the gastrocnemius, the proximal branch to the soleus and the nerve to the plantaris muscle. Out of 19 instances the nerve to the plantaris was the most proximal branch in 9, the nerves to the gastrocnemius in 9, and in one instance the nerve to the plantaris, in conjunction with the branch to the lateral head of the gastrocnemius and the proximal nerve to the soleus. Usually the nerve to the medial head of the gastrocnemius arises slightly proximal to that to the lateral head. The latter arises near or in conjunction with the proximal nerve to the soleus muscle.

Next distal to the nerves to the plantaris and gastrocnemius muscles and the proximal nerve to the soleus arise the nerves to the popliteus and posterior tibial muscles. These nerves often arise from a common branch. When they arise separately the nerve to the poplitus is the more proximal in the great majority of instances.

Next distal usually comes the distal nerve to the soleus, although this nerve may arise proximal to the nerve to the tibialis posterior or in conjunction with this. Then follow the nerves to the flexor digitorum longus and to the flexor hallucis longus. The two latter frequently arise from a common trunk which may also be combined with the distal nerve to the soleus. The nerve to the flexor hallucis is almost always the most distal in origin of the nerves under consideration, but occasionally a distal branch to the flexor digitorum longus has a more distal origin (in two instances out of 34).

c. Relation to One Another of the Nerves to the Muscles.

Nerve to plantaris.—In all but one out of 21 instances the nerve to the plantaris muscle arose independently from the tibial nerve. In this instance it arose in conjunction with the nerve to the lateral head of the gastrocnemius and the proximal nerve to the soleus muscle. In one instance two nerves could be traced to the plantaris.

Nerve to medial head of gastrocnemius.—In one instance out of 35 two separate parallel branches passed into this head. Occasionally near its origin from the tibial nerve the nerve to the medial head of the gastrocnemius is bound up for a short distance with that to the lateral head.

Nerve to the lateral head of the gastrocnemius.—Out of 35 instances in 20 this nerve arose independently or in conjunction with that to the medial head from the posterior tibial; in 14, in conjunction with the proximal nerve to the soleus and in one in conjunction with the proximal nerve to the soleus and the nerve to the plantaris.

Proximal branch to soleus.—Out of 35 instances in 20 this branch arose independently, in 14 it arose in conjunction with the nerve to the lateral head of the gastrocnemius, and in one in conjunction with the nerve to the lateral head of the gastrocnemius and the nerve to the plantaris.

The above nerves form a group, the trunks of which may be more or less united with one another, but not with those of the following set.

Nerve to the popliteus.—This nerve arose independently in 15 out of 26 instances. In 10 instances it arose in conjunction with the nerve to the tibialis posterior and in one, with the distal nerve to the soleus and the nerve to the tibialis posterior. In two instances a secondary branch entered the superior edge of the muscle.

Halbertsma, 47, described a nerve which arises in the popliteal space, gives rami to the popliteus and posterior tibial muscles, and is continued distally, partly in the substance of the interosseous membrane, to the inferior tibio-fibular articulation. It gives branches to the superior tibio-fibular articulation, to the tibia and the interosseous membrane. When the nerves to the popliteus and tibialis posterior arise separately this nerve is continued distally either from nerve to the popliteus or, more rarely, from that to the tibialis posterior. McMurrich, 04, considers this branch to represent the important ramus profundus of amphibia and reptiles. This supplies the deep muscles of the plantar surface of the crus and is continued into the foot as the internal plantar nerve. In the lower mammals it ends at the ankle. In man another nerve arises from the nerve to the deep muscles and passes distally along the course of the peroneal vessels to the ankle. It gives branches to the shaft of the fibula and the medullary artery.[24]

Nerve to the tibialis posterior.—Out of 38 instances in 20 the nerve arose independently, in 5 it arose in conjunction with the nerve to the popliteus. In 5 instances it arose in two branches, one of which in each instance was associated with the nerve to the popliteus while the other in one instance was independent, in one was associated with the nerve to the flexor digitorum longus and in three with the distal nerve to the soleus muscle. In 4 instances the nerve to the tibialis posterior was associated with the distal branch to the soleus, in 3 with the nerves to the flexor digitorum longus and flexor hallucis longus muscles, and in one instance with the distal nerve to the soleus and with the nerve to the flexor digitorum and flexor hallucis.

Distal nerve to the soleus.—Out of 37 instances, in 20 this nerve arose independently. In 7 it arose in conjunction with a nerve to the posterior tibial muscle; in 4, in conjunction with one to the flexor digitorum longus

[24] Rauber, cited by G. D. Thane, Quain's Anatomy, 10th ed.

and flexor hallucis longus muscles; in 3, in conjunction with the nerve to the flexor hallucis muscle; in one in conjunction with that to the flexor digitorum; in one, in conjunction with that to the popliteus and posterior tibial muscles; and in one in conjunction with a nerve to the posterior tibial, flexor digitorum and flexor hallucis muscles.

Nerve to the flexor digitorum longus. Out of 36 instances, in 20 the nerve arose independently, in 6 of these by two separate branches; in 6 instances it arose in conjunction with the nerve to the flexor hallucis muscle; in 4 others, in conjunction with this and the distal nerve to the soleus. In three instances it arose in conjunction with the nerve to the flexor hallucis and that to the tibialis posterior muscle; in one, in conjunction with that to the tibialis posterior; in one, in conjunction with the distal nerve to the soleus; and in one, with the nerve of the tibialis posterior and flexor hallucis and the distal nerve to the soleus.

Nerve to the flexor hallucis muscle.—Out of 35 instances, in 18 the nerve arose independently, in two of these by two separate branches. In six instances the nerve arose in conjunction with the nerve to the flexor digitorum longus muscle; and in 4 other instances, in conjunction with this and the distal nerve to the soleus. In three instances it arose in conjunction with the distal nerve to the soleus; in three, in conjunction with the nerve to the flexor digitorum and tibialis posterior; and in one, in conjunction with the distal nerve to the soleus and the nerves to the tibialis posterior and flexor digitorum longus.

4. Development and Innervation of the Muscles Supplied by the Lateral Plantar Nerve.

To this group belong the quadratus plantæ, the abductor, flexor brevis, and opponens digiti quinti, the interossei, and the three lateral lumbrical muscles.

M. quadratus plantæ.—The anlage of this muscle appears in a 14 mm. embryo (Plate IX, Fig. 4) medial to the lateral plantar nerve as this curves about the tuber calcanei. Schomburg, [00], describes it in about the same position, but fused with the flexor hallucis longus at a nearly corresponding stage. In the 14 mm. embryo the nerve to the muscle is not distinct but in a 20 mm. embryo (Plate IX, Fig. 6) a well marked nerve enters its superficial surface from the deep surface of the lateral plantar nerve as this crosses the muscle. The muscle can readily be traced from the calcaneus to the deep surface of the plantar aponeurosis.

In the adult the nerve to the quadratus plentæ arises from the lateral plantar nerve near the medial margin of the muscle and crosses on or

near the superficial surface of the muscle about midway between its origin and insertion and parallel with the tendon of the flexor digiti quinti longus. I have never seen the nerve for this muscle arise from the medial plantar nerve as described in the anatomy of Poirier and Charpy.

In the adult this muscle is frequently reinforced by a fasciculus which may arise from either of the bones of the crus, from one of the deeper muscles of the crus, from the deep muscle fasciæ, or from the calcaneus, Le Double, 97. The muscle may be inserted into any of the digital tendons, but most frequently into the 2d, 3d, and 4th; into that to the 5th toe less frequently; into that to the great toe rarely.

McMurrich, 04, on phylogenetic grounds thinks that the quadratus plantæ is differentiated from the distal end of the same deep layer of crural muscles from which are derived the tibialis posterior and the flexor digitorum (tibial flexor). Schomburg, on the other hand, considers it more intimately related to the flexor fibularis, a point of view strengthened by the fusion which he found between the quadratus plantæ and the flexor hallucis longus in a young embryo. As mentioned above, I did not find this connection in the 14 mm. embryo. Nor does the nerve supply of the muscle indicate a close union between it and the tibialis posterior or either the tibial or the fibular flexor.

The quadratus plantæ "is clearly represented in the lacertilia where it is supplied by a branch of the ramus profundus." (McMurrich, 04).

In monotremes it arises from the calcaneus (Leche). In the majority of marsupials it is probable that it exists in a rudimentary condition (McMurrich). In edentates it is absent in some forms, well marked in others. In some insectivora it is fused with the abductor metacarpi digiti minimi. In the higher mammals it is absent in some forms and well developed in others (*i. e.*, dog and cat). In some apes it is fused with the flexor digitorum tibialis (Leche).

M. abductor digiti quinti.—In the 14 mm. embryo (Plate IX, Fig. 4) the anlage of this muscle may be seen immediately distal to the tuber calcanei and lateral to the n. plantaris lateralis. In an embryo of a corresponding age Schomburg, 00, pictures the muscle as extending to the 4th metatarsal, but I have found no corresponding condition in the embryos I have studied. In embryo XXII, length 20 mm. (Plate IX, Fig. 6) the muscle extends to the base of the 5th metatarsal and has a more lateral position than in the 14 mm. embryo. At this stage a nerve may be seen extending into the medial margin of the muscle from the deep surface of the lateral plantar nerve.

In the adult the muscle is developed medially so as partially to overlap the lateral head of the m. quadratus plantæ. It varies greatly in structure. The main bulk of the fibre bundles usually extends somewhat obliquely from the calcaneus, the plantar fascia and the tendinous aponeurosis on the lateral side of the muscle near its origin to a tendon

which extends high on the medial side of the deep surface of the muscle. Fibre bundles may also run from the calcaneus to the tuberosity of the fifth matatarsal and from this to the tendon of insertion. The more lateral and distal fibre bundles are those least frequently developed.

The nerve may be distributed either near the deep or near the superficial surface of the muscle. The former appears to be the case when the muscle is slightly developed. The chief muscle branches then extend across the middle third of the constituent muscle bundles near the deep surface. In case the calcaneo-metatarsal bundles are well developed a special branch may be sent to these. When the muscle is well developed the nerve enters the proximal margin of the muscle and its chief branches extend across the middle third of the more superficial muscle bundles finally terminating in those most distal bundles which lie on the lateral side of the fifth metatarsal. Other modes of distribution are also found but they agree in general features with those described.

Flexor brevis and opponens digiti quinti.—Beyond the anlage of the abductor digiti quinti the lateral plantar nerve in the 14 mm. embryo (Plate IX, Fig. 4) lies superficial to an ill-defined mass of tissue in which no segmentation into muscles can be made out. In the 20 mm. embryo (Plate IX, Fig. 6) a nerve branch extends from the lateral plantar nerve to the base of the 5th metatarsal and near the tip of this two slightly defined areas of partially differentiated tissue probably represent the anlages of the two muscles under consideration. According to Schomburg the anlage of these muscles lies at first in the area between the 4th and 5th metatarsals but for this statement I find no support in the embryos studied. According to Ruge, 78, and Schomburg, 00, the flexor brevis and opponens muscles arise from a common anlage which becomes later differentiated into the two muscles.

In the adult a single nerve is commonly distributed across the middle third of the bellies of each muscle.

Mm. interossei.—Ruge, 78, called attention to the fact that the interosseous muscles with the possible exception of the first dorsal have a plantar origin and that later the dorsal interossei wander between the metatarsals to the dorsal surface. Schomburg, 00, has confirmed this observation and has also shown that the dorsal interosseous I is originally plantar in position. In later embryonic stages Schomburg describes the dorsal interosseous II as extending on the plantar surface somewhat like the plantar interossei while the plantar interosseus I shows a tendency to wander dorsally like a dorsal interosseous.

The first signs of the interossei muscles which I have seen are ill-de-

fined anlages in an embryo of 20 mm. (Plate IX, Fig. 6). To the proximal extremity of each anlage branches are given from the lateral plantar nerve. The later stages of development I have not followed out carefully.

M. adductor hallucis.—This arises, as pointed out by Ruge, from an anlage at the base of the 2d metatarsal and from here wanders into its adult position. The anlage of the muscle is shown in Plate IX, Fig. 6. The later development of the muscle I have not followed. According to Ruge, 78, the transverse head of the adductor comes from the same anlage as the oblique, while Schomburg, 00, considers that the latter muscle arises from a separate anlage. According to Poirier the nerves of the two portions of the adductor arise from a common trunk which would be in favor of Ruge's view. I have found the nerves arising usually from quite distinct branches of the lateral plantar nerve. One nerve enters the caput obliquum near the proximal end of the middle third; and the other, the caput transversum near its centre.

Lumbricales.—In neither embryo CXLIV, length 14 mm., nor in embryo XXII, length 20 mm., are the lumbricales clearly differentiated. In the latter embryo, Plate IX, Fig. 5, however, the anlage of the lumbrical muscle of the 2d toe is just beginning to appear and to it a slight nerve twig may be traced from the medial plantar nerve. As pointed out by Schomburg the lumbrical muscles appear during the second half of the second month as separate anlages near the distal extremity of the metatarsal bones and from here wander toward their attachments to the tendons of the flexor digitorum longus. The three lateral lumbrical muscles were found supplied by the lateral plantar nerve and the medial by the medial plantar in 9 out of 10 instances by Brooks, 87, while the two medial muscles were supplied both by the medial and lateral plantar nerves in one instance. He considers that the lumbrical muscles belong primitively to the medial plantar territory.

5. Development and Innervation of the Muscles Supplied by the Medial Plantar Nerve.

To this group belong the flexor digitorum brevis, abductor hallucis, the flexor hallucis brevis and the medial lumbrical muscle. This last has been considered in connection with the muscles of the preceding group.

M. flexor digitorum brevis.—This muscle develops comparatively late. In the 14 mm. embryo I have been able to determine no distinct signs of the muscle. In a 20 mm. embryo (Plate IX, Fig. 5) the anlage of the

muscle may be made out on the surface of the aponeurosis of the long flexor muscles above the region of the middle cuneiform bone. Differentiation is just beginning so that no distinct muscle fibres may be made out. A small nerve may be traced into its medial margin. The tendons have not begun to develop. Soon after this stage the muscle undergoes rapid development. Proximally it extends to the tuber calcanei, distally it sends forth tendons to the toes.

In the adult the chief variations are those marked by a reduction of the muscle, especially that portion belonging to the fifth toe. The muscle is supplied by a nerve which enters the medial margin.

M. abductor hallucis.—This muscle is not distinctly visible in embryo CXLIV (length 14 mm.). It can be distinguished in embryo XXII (length 20 mm.), although differentiation is not here well marked. (Plate IX, Fig. 6). The muscle arises on the medial edge of the plantar surface of the foot over the navicular, first cuneiform, and the base of the 1st metatarsal bones and at a considerable distance from the tuber calcanei. It arises in close association with the m. flexor hallucis brevis. With the torsion of the foot which carries the calcaneus in a medial direction the anlage of the abductor extends proximally to be attached to the tuber calcanei.

In the adult a branch from the medial plantar nerve usually enters near the middle of the lateral border of the muscle. The relation of the nerve to the muscle anlage in embryo XXII is shown in the figure.

Flexor hallucis brevis.—Like the other muscles of this group this muscle is not distinguishable in embryo CXLIV, length 14 mm. Even in embryo XXII, length 20 mm. (Plate IX, Fig. 6), it is only beginning to appear. The cells of the anlage are closely packed together. To the anlage a nerve branch is given. I find the anlage somewhat more medially placed on the base of the first metatarsal than that shown by Schomburg, 00. The anlage is incompletely divisible into two portions, a medial and a lateral. During further development the lateral belly approaches the adductor hallucis. The medial belly in embryo XXII is associated with the abductor hallucis, although according to Schomburg it is brought into association with this muscle later than the lateral head is brought into association with the adductor hallucis.

In the adult the nerve enters between the two bellies of the muscle and spreads out into branches which pass between the constituent muscle bundles. It is only rarely that the lateral head of the muscle is supplied by the lateral plantar nerve.

Comparative anatomy of the intrinsic plantar muscles.—According to McMurrich, 04, the muscles of the crus terminate primarily at the ankle either on the plantar aponeurosis or the tarsus. The tendons whereby the long flexors of the toes are attached to the digits he looks upon as a differentiation of a deep plantar aponeurosis. According to this view the foot, in which but one set of crural muscles is attached through tendons to the digits, is to be looked upon as more primitive than the hand, in which superficial and deep forearm flexors are thus attached. In the foot there are to be distinguished several layers of intrinsic muscles, the more superficial of which, the flexor digitorum brevis and the lumbricales, arise in man from or in connection with the plantar aponeurosis or its derivatives, while the deeper layers arise from the tarsus and metatarsus.

The deeper intrinsic muscles of the hand and foot are considered by Cunningham, 82, and Brooks, 87, to have been derived from three primary layers, a superficial layer of four muscles primarily adductors, an intermediate layer of bicipital short flexors, one for each digit, and a deep layer of six abductors. The lateral plantar nerve crosses between the superficial and the intermediate layer.[25]

McMurrich, 03, differs greatly from Cunningham in the layers to which he would ascribe the muscles of the hand. Thus he recognizes the following layers:

Flexor brevis superficialis: Palmaris brevis, abductor digiti quinti, opponens digiti quinti, flexor brevis digiti quinti, abductor pollicis, opponens pollicis, flexor pollicis brevis.

Flexor brevis medius, stratum superficiale: The lumbricales.

Flexor brevis medius, stratum profundum: The adductor pollicis.

Flexor brevis profundus: The interossei volares, interossei dorsales (in part).

Intermetacarpals: The interossei dorsales (in part).

McMurrich has not yet published his paper on the phylogeny of the muscles of the foot, so that his views as to the origin of these muscles cannot be given, but doubtless the layers there, from his point of view, resemble those of the hand.

The subject of the comparative anatomy of the plantar muscles is too intricate to be entered upon here at length. Leche gives a brief summary of the conditions found in the mammalian series.

From the standpoint of embryological development the division of the deep plantar muscles adopted by Ruge, 78, is of the greatest value. He recognizes a medial group consisting of the abductor and the flexor brevis hallucis, innervated by the medial plantar nerve, and two groups innervated by the lateral plantar nerve, a more superficial group of "contrahentes" which lie plantarwards from the deep branch of the nerve and a group of "interossei" which lie deeper than this nerve. He also points out that in many of the mammals the interossei have permanently a plantar position which corresponds with the early embryonic condition in man.

[25] See Quain's Anatomy 10th ed., Vol. II, Pt. II, p. 276.

6. *The Muscle Branches of the Plantar Nerves.*

While it is certain that the set of spinal nerves supplying the lateral plantar nerve as a group are more distally situated than those supplying the medial plantar nerve, the difficulties of tracing the nerves supplied to the muscles of the sole back to the sacral plexus make it impossible at present to give the spinal nerve supply of these muscles.

Compared with the other nerves of the leg the plantar nerves seem to be unusually constant in their mode of distribution of branches. The difficulties of accurate dissection of the nerves of the intrinsic muscles of the sole of the foot, however, make it more difficult than in other regions to utilize the work of students in getting reliable charts of this nerve supply. In general the descriptions given in the various anatomies agree pretty well. In the anatomy of Poirier and Charpy the supply of the quadratus plantæ is given as coming from the medial plantar. In a large number of dissections which I have followed this branch arose in every case from the lateral plantar, usually proximal but sometimes distal to the branch to the abductor of the fifth toe. This is the situation usually described for it in the text-books. The dissections which I have followed also serve to substantiate the statement given in Quain's Anatomy (10th edition) that the lateral plantar nerve only occasionally gives a branch to the lateral head of the m. flexor brevis hallucis and to substantiate the statement of Brooks, 87, that in about one in ten instances the medial as well as the lateral plantar nerves supply both the first and second lumbrical muscles. There is, however, considerable variation in the way in which the different nerves to the interosseous and lumbrical muscles and the transversales pedis are bound for a distance in common trunks.

XI. SUMMARY AND CONCLUSIONS.

The intrinsic musculature of the inferior extremity in man is differentiated from the blastema of the limb-bud. No processes from the myotomes are sent into the limb from the lumbar or sacral myotomes. After the differentiation of the myotomes from the somites the myotomes are bounded on the external surface, the sides and ends by a clearly marked membrane which is retained until after the lumbo-sacral nerves have extended well into the limb-bud.

Soon after the lumbo-sacral spinal nerves begin to extend into the limb-bud tissue differentiation takes place in the blastema of the bud.

At the center a core of scleroblastema, on each side of this a thick layer of myoblastema, at the periphery of the limb-bud a thinner layer of dermoblastema are differentiated. This differentiation begins near the anlage of the hip joint and extends proximally and distally.

The myoblastema represents the anlage of the muscles and of the skeletal framework of the musculature, including the fasciæ and the tendons.

The spinal nerves which grow into the limb-bud fuse to form a plexus and from this the nerves of the limb arise. At the time these nerves extend distally and give off branches the myoblastema becomes differentiated into anlages for specific groups of muscles and each of these anlages becomes further differentiated into the anlages of the specific muscles which compose the group. The main nerve trunks grow as a rule in regions which lie between the anlages of muscle groups, the main branches to each of the groups between the anlages of the muscles which constitute the group, and the intramuscular branches in the intramuscular septa which appear between the differentiating bundles of muscle fibres. Finally the terminal branches for the individual muscle fibres are given off. The site of entry of a nerve marks the region of earliest differentiation in the muscle. In many instances, at least, the distribution of the nerve in an adult muscle indicates the course of development of that muscle. (Nussbaum, 94).

The development of muscles from the muscle anlages consists essentially of a differentiation of the, at first, apparently nearly homogeneous tissue of the anlage, into muscle cells and into the connective tissue framework which serves to hold these in place and harness them to the structures on which they are to act. The adult architecture of a muscle must be understood before its development can be intelligently followed.

In the simplest muscles in the adult the muscle fibres are bound by the endomysium into bundles which are inserted at each end of the muscle into a tendon. As a rule neither the muscle-fibres nor individual bundles of fibres extend the entire distance from tendon to tendon.[26] The fibre bundles anastomose in such a way that they form a long-meshed network, such as that diagrammatically represented in Fig. 7 b.

The muscle-fibres either take a nearly parallel course from one tendon to the other, Fig. 7 c, or they diverge from one tendon toward the other, Fig. 7 d. In the majority of simple muscles the distance from tendon

[26] In some short mammalian muscles, like the segments of the rectus abdominis of the mouse, the muscle-fibers run from tendon to tendon. On the segmental musculature of elasmobranches and urodeles, see Bardeen, 03.

to tendon along lines parallel with the muscle fibres is approximately the same in all parts. There are, however, numerous exceptions, the most marked of which are found in larger sheet-like muscles such as the oblique and transverse muscles of the abdomen. Frequently in case of exceptions of this nature, as for instance in case of the abdominal muscles, the adult

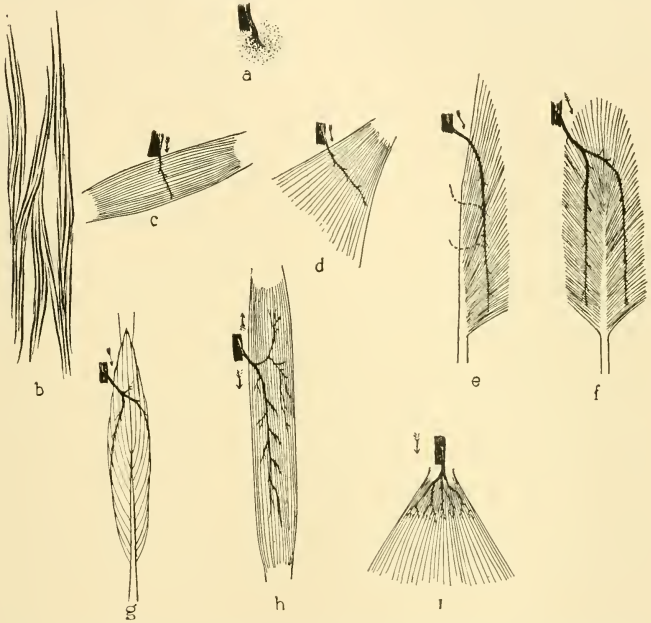


Fig. 7. Diagrams to illustrate nerve-muscle development. *a*. Embryonic muscle anlage. *b*. Anastomosing bundles of muscle fibers. *c*. Band-like muscle developed transverse to course of main nerve trunk. *d*. Triangular muscle developed transverse to course of main nerve trunk. *e*. Pennate muscle developed parallel with course of main nerve trunk. *f*. Bipennate muscle developed parallel with course of main nerve trunk. *g*. Fusiform muscle. *h*. Band-like muscle developed parallel with course of main nerve trunk. *i*. Triangular muscle developed in direction with course of main nerve trunk.

human muscle represents a combination of several simpler muscles in each of which the general rule holds good in the embryo or in some of the lower mammals. For the architecture of the abdominal muscles in the mammals, see Bardeen, 03.

Since the more complex muscles are usually capable of being analyzed into parts the structure of which resembles that of the simpler muscles, we shall consider here the development merely of several simpler types of muscle. The structural units of the more complex muscles develop in a similar manner.

The nerve usually enters the anlage of a muscle near the center of the side toward the main trunk from which its special nerve arises, Fig. 7 a. The relation of the chief branch or branches of the nerve of the muscle to the fibre-bundles depends on whether the course of the muscle fibres is transverse to or parallel with the main trunk from which the nerve arises.

If the fibre bundles of the muscle take a direction directly or obliquely transverse to the course of the main nerve trunk the nerve to the muscle, or its chief branches, usually passes for some distance across the fibre bundles about midway between the tendons and give off rami on each side from which in turn an intramuscular nerve plexus arises, Fig. 7 c-g. The direction of the course of the main nerve branches on or in an adult muscle of this nature indicates the course of growth of the muscle from the anlage in a direction transverse to the long axis of the muscle fibres. This growth is relatively slight in case of ribbon-like muscles, Fig. 7 c, somewhat greater in case of triangular muscles, Fig. 7 d, and extensive in case of muscles like the intercostal muscles and pennate or bipennate muscles, Fig. 7, e, f. Two or more branches may enter muscles of these latter types at different levels from the main nerve trunk, dotted lines Fig. 7 e.

It is at first difficult to recognize that in most fusiform muscles the distance from tendon to tendon along the course of the muscle-fibres is approximately equal. The course of the muscle-fibres in such muscles is diagrammatically represented in Fig. 7, g. It will be noted that the course of the chief branches of the nerve to the muscle is approximated midway between the tendons to which the fibre-bundles are attached.

If the long axes of the fibre-bundles of a muscle are developed in a direction somewhat parallel with the course of the main trunk from which the chief branches to the muscle arise, the branches usually enter the proximal third of the belly of the muscle and extend distally parallel with the muscle fibres, at the same time giving off rami from which an extensive intramuscular plexus is formed. The course of the chief rami within a muscle of this type indicates the course of growth of the muscle in a direction parallel with the muscle fibres. See Fig. 7, h and i.

Metameric segmentation in the innervation of the limb muscles is due

not to the ingrowth into the limb of myotomes accompanied by nerves, but to the fact that a given region in the developing musculature is in the more direct path of fibres extending into the limb from one or two specific spinal nerves. The number of spinal nerves contributing to the innervation of the inferior extremity in man varies from six to nine, the number contributing to the innervation of the musculature probably varies from five to eight. The number as well as the position of the spinal nerves serving to innervate a given muscle varies greatly in different individuals.

With a few exceptions it is difficult or impossible to trace back to their origin from the plexus the fibres composing the nerve of supply of a given muscle in the inferior extremity in man. The path of fibre bundles in a nerve is quite different from that of the nerve fibres composing the nerve. The connective tissue which serves to hold together the nerve fibres and to distribute blood vessels to the nerve does not form continuous sheets about continuous bundles of nerve fibres. On the contrary it forms enveloping layers which are continued for but a short distance about a given group of fibres and then breaks up and becomes fused with similar enveloping sheets about other groups of fibres. The nerve fibres take a much more direct course in a nerve than any bundles that can be dissected from the nerve. A study of the origin of the branches of a nerve and the variation in the relation of these branches to one another makes it possible to construct a schematic cross section of a nerve trunk in which the relations of the nerve fibres in the trunk are more accurately revealed than in mere dissection of the branches back into the component fibre bundles of the nerve. On pages 308, 316, and 322 I have shown such schematic diagrams of the femoral, obturator and sciatic nerves. The nerve fibres of contiguous areas may branch off in a common trunk, but nerve fibres in discontinuous areas never do. On Plate III I have shown schematically the probable regions occupied by the fibres destined for the chief branches of the main nerves of the inferior extremity in the nerve trunks near the pelvis at the period when the segmental relations of the spinal nerves to the limb are becoming established.

In the adult nerves variation is frequent and extensive. The main nerve trunks are fairly constant in position, the greatest variation being found in the course of the peroneal nerve in the thigh. This nerve is frequently separated from the tibial nerve by a part or the whole of the piriformis muscle. In one instance I have seen it separated by a part of the short head of the biceps, p. 293. In the embryo the peroneal and

tibial nerves in the thigh are separated by a considerable amount of dense tissue.

There is much variation in the number and position of the spinal nerves which supply the main nerve trunks of the limb as well as in those which supply the smaller branches which pass directly from the plexus to the gluteal muscles and the piriformis. There is also great variation in the number, course and distribution of the branches which pass from the main nerve trunks to the muscles and the skin. No correlation has been discovered between variation in the source of supply and variation in peripheral distribution of the intrinsic nerves of the limb, with the exception of the cutaneous border nerves. No marked correlations have been discovered between either sort of variation and race, sex, or side of body.

While the development of the musculature is fairly direct, there is probably as much correlation between the ontogeny and phylogeny of the muscles of the leg as between the ontogeny and phylogeny of the skeleton.

D. PERINEAL MUSCULATURE AND THE NERVES OF THE PUDIC GROUP.

a. Embryonic Development.

In an embryo of 11 mm. (Plate III, Fig. 1) the sacral plexus is fully formed and several branches may be seen extending out toward the cloaca and viscera. These branches indicate the developing pudic and visceral nerves, but differentiation has not proceeded sufficiently far to make it possible to determine with certainty what each of the branches represents. The myotomes of the sacro-coccygeal region are distinct. No specific differentiation of the perineo-caudal musculature is apparent. The relations of the pubic nerves to the nerves of the leg are shown schematically in Plate III, Fig. 3.

In a slightly older embryo (Plate X, Fig. 1) the main branches of the pudic and visceral nerves have appeared. The dorsal nerve of the penis arises in the main from the 3d sacral nerve. The perineal nerves arise from the (2d), 3d, and 4th sacral nerves. The hemorrhoidal nerve arises from the 3d and 4th sacral nerves. About the region of the cloaca there is some condensation of tissue, but there is no distinct differentiation of muscle. From the 3d and 4th sacral nerves branches are given to a highly developed visceral plexus in which a large amount of chromophile tissue is apparent. This tissue mass lies lateral to the intestine and extends

nearly to the urachus. Anteriorly it is continued into a similar mass extending down from the region of the suprarenal gland.

In company with the visceral branch from the 4th sacral nerve there arises a nerve which extends out into a differentiating mass of tissue which probably represents the levator ani muscle. There is no good evidence to show that this muscle arises from the myotomes. The coccygeal musculature which lies dorsal and lateral to the levator ani seems, however, evidently to arise from the ventral tips of the caudal myotomes. Into it extend nerves from the 4th and 5th sacral and possibly from the caudal nerve. This, as also in embryo CIX, is relatively at this stage very large.

In embryo XXII, length 20 mm. (Plate X, Fig. 2) conditions similar to those just described may be found. The direction in which the sections are cut makes a reconstruction of the region somewhat imperfect. The results have been controlled by study of another somewhat older embryo, CXLV, length 33 mm. The plexus is of a more anterior type than that of 144. The dorsal nerve of the penis and perineal nerves apparently arise largely from the 2d sacral nerve and the 4th sacral nerve seems not to enter into the pudic plexus. The perineal musculature is undergoing specific differentiation, but no attempt has been made to determine definitely the boundaries of the various muscles. The levator ani muscle is well differentiated. The visceral plexus is even more extensive than in the preceding stage.

For comparison of embryonic conditions with the distribution of the pudic nerves in the adult male, the well-known illustration of Hirschfeld and Léveillé may be used. It is to be noted that previous to the outgrowth of the pudic nerves the cloaca and urachus occupy a more distal position relative to the spinal column (Fig. CIX) than they do at the period when these nerves are developed (Plate X, Figs. 1 and 2). Later, the external genitalia shift again distally.

The paths taken by the growing nerves are fairly direct. That of the dorsal nerve of the penis is most so. The perineal nerves bend more in a distal direction. The nerve of the levator ani muscle takes a course at right angles to the path of the main trunk from which it arises. It may readily be seen that the most anterior root fibres of the pudendal nerve enter the dorsal nerve of the penis, the most posterior the hæmorrhoidal nerve. Cutaneous branches also arise from the caudal nerve.

According to Popowsky, 99, at a period when the cloaca is still present a sheet of muscle forms a sphincter around its opening. Later, when the rectal becomes separated from the urogenital portion of the cloaca the sphincter is divided, the posterior portion becoming the sphincter ani

TABLE

Types of Origin of Pudic Nerves from Spinal Nerves.			Frequency of		
			A	B	C
N. Pudendus.	Separate N. Hæmorrhoidalis.	Separate N. Dorsalis Penis.	XXIV XXVI	XXIV XXVII	XXIV XXVIII
XXVI, XXVII			1	1	
XXVII				2	
XXVI, XXVII	XXVIII			1	
XXVII	XXVIII			1	
XXVI, XXVII, XXVIII				2	4
XXVII, XXVIII		XXVII[27]		11	37
XXVII, XXVIII	XXVIII			3	1
XXVIII					1
XXVI, XXVII, XXVIII	XXVII, XXVIII, XXIX				1
XXVII, XXVIII	XXVIII, XXIX	XXVII, XXVIII[28]		1	2
XXVII, XXVIII, XXIX		XXVII[29]		1	7
XXVIII, XXIX	XXVIII, XXIX	XXVIII[30]			
XXVIII, XXIX		XXVIII[31]		1	8
XXVIII, XXIX	XXX				
XXVIII, XXIX, XXX					
Number of Instances			1	24	61

[27] In three instances: W. F. R; W. F. L; W. M. R.
[28] In one instance: W. M. R.
[29] In two instances: W. M. L; B. M. L.
[30] In two instances: B. F. R; B. M. L.
[31] In one instance: B. M. R.

XXIX.

Association with Various Types of Plexuses.					Race, Sex, and Side of Body.							
D	E	F	G		White.				Negro.			
XXIV	XXIV, XXV	XXIV	XXIV, XXV	Furcal N.	Male.		Female.		Male.		Female.	
XXVIII	XXVIII	XXIX	XXIX	Most Distal Nerve to Limb.	R	L	R	L	R	L	R	L
				No. of Inst.								
				2						1		1
				2		1						1
				1	1							
				1		1						
1				7	2	1			2	2		
34	3			85	13	10	1	4	20	18	11	8
				4			1		2		1	
3				4			1				2	1
				1	1							
1	1			5	1	2				1	1	
22	1	5	3	39	6	7	1		8	6	3	8
5	1	1		7					2	2	2	1
37	8	6	15	75	7	6	2	1	18	20	11	10
		1		1						1		
			1	1					1			
103	19	13	14	235	31	28	6	5	53	51	31	30

TABLE XXX.

Type of Plexus from which the Chief Nerve to the M. Levator Ani arises.				Frequency of Origin of Nerve to M. Levator Ani from:					
Type.		Furcal Nerve.	Most Distal Spinal Nerve to Limb.	Nn. Sp. XXVII XXVIII	Nn. Sp. XXVIII	Nn. Sp. XXVIII XXIX	Nn. Sp. XXIX	Nn. Sp. XXIX	Total Number.
	A	XXIV	XXVI						
Ant.	B	XXIV	XXVII	1	4				5
	C	XXIV chiefly to sacral plexus	XXVIII		9	3	14		26
Norm.	D	XXIV chiefly to lumbar plexus	XXVIII		1	4	29		34
	E	XXIV-XXV, or XXV	XXVIII		1	1	5		7
Post.	F	XXIV	XXIX			1			1
	G	XXIV-XXV, or XXV	XXIX			1	6	1	8
Total Number.....				1	15	10	54	1	81

TABLE XXX.—*Continued.*

Race, Sex, and Side of Body.			Frequency of Origin of Nerve to M. Levator Ani from:					
			Nn. Sp. XXVII XXVIII	Nn. Sp. XXVIII	Nn. Sp. XXVIII XXIX	Nn. Sp. XXIX	Nn. Sp. XXIX	Total Number.
White.	Male.	R	1	1	3	5		10
		L		4	1	7		12
	Female.	R		2		2		4
		L		3		1		4
Negro.		R			4	10		14
		L		1	1	14		16
		R		2		6	1	9
		L		2	1	9		12

while the anterior portion becomes differentiated into the various perineal muscles. The details of this process I have not followed.

For the comparative anatomy of the perineal muscles, see H. Eggeling, 96, M. Holl, 96.

b. Nerve Variation in the Adult.

There is considerable variation in the origin and distribution of the nerves of the perineal region in man. Commonly the hæmorrhoidal and the perineal nerves and the dorsal nerve to the penis (clitoris) are branches of a common trunk, the pudic nerve, which arises from the 27th and 28th spinal (2d and 3d sacral) or the 28th and 29th spinal (3d and 4th sacral) nerves. The origin may, however, be from the 26th and 27th; the 27th; the 26th, 27th, and 28th; the 28th; the 27th, 28th, and 29th; or from the 28th, 29th, and 30th spinal nerves. In the accompanying table the frequency of these various modes of origin is shown.

Not infrequently (in 20 out of 235 instances, 8.5%) the hæmorrhoidal branch has a separate origin from the plexus, usually from the 28th, or 28th and 29th spinal nerves.

Less frequently the dorsal nerve of the penis (clitoris) has an independent origin (in 9 out of 235 instances, 3.9%). In such instances the nerve arises from the 27th; 27th and 28th; or 28th spinal nerves (see Table XXIX).

The chief nerve to the levator ani muscle arises usually in conjunction with viscereal branches from the 29th spinal (4th sacral) nerve. It may arise from the 27th and 28th; the 28th; the 28th and 29th; or the 29th and 30th spinal nerves (see Table XXX). Other small branches are also frequently given to this muscle.

The nerves to the coccygeus muscle arise from the last spinal nerve contributing to the pudic plexus and also usually from the next more distal spinal nerve.

The visceral branches usually arise from the last two spinal nerves entering the pudic plexus and also often from the next most distal spinal nerve.

The charts which I have show great variation in the peripheral course and distribution of all of these nerves. The difficulties of making thoroughly accurate dissections and charts of the nerves of the perineal region make it seem inadvisable to try to use these charts for statistical purposes. In general the distribution corresponds with that given in the anatomies of Poirier and Charpy and Quain, and with those pictured in the atlases of Toldt and Spalteholz.

LITERATURE.

Bardeen, C. R.—The development of the musculature of the body-wall in the pig, including its histogenesis and its relations to the myotomes and to the skeletal and nervous apparatus. Contributions to the Science of Medicine, dedicated to W. H. Welch, 1900. The Johns Hopkins Hospital Reports, 1900.

——— A statistical study of the abdominal and border nerves in man. This Journal, Vol. 1, p. 203, 1902.

——— The growth and histogenesis of the cerebro-spinal nerves in mammals. This Journal, Vol, 2, pp. 231-257, 1903.

——— Variations in the internal architecture of the M. obliquus abdominus externus in certain mammals. Anat. Anz., Vol. 23, p. 241, 1903.

——— The bimeric distribution of the spinal nerves in elasmobranchii and urodela. This Journal, Vol. 3, p. v, 1904.

——— Studies of the development of the human skeleton. This Journal, Vol. 4, pp. 265-302, 1905.

Bardeen, C. R., and Elting, A. W.—A statistical study of the variation in the formation and position of the lumbo-sacral plexus in man. Anatomischer Anzeiger, Vol. 19, p. 124, 1901.

Bardeen, C. R., and Lewis, W. H.—Development of the back, body-wall, and limbs in man. This Journal, Vol. 1, p. 1,, 1901.

v. Bardeleben, C.—Hand und Fuss. Verhandlung der anatomischen Gesellschaft, 1904.

Bolk, L.—Beziehungen zwischen Skelet, Musculatur, und Nerven der Extremitäten, dargelegt am Beckengürtel, an dessen Muskulatur, sowie am Plexus lumbo-sacralis. Morpholog. Jahrbuch, Bd. 21, pp. 241-277, Bd. 22, pp. 357-379, 1894.

——— Die Segmentaldifferenzierung des menschl. Rumpfes und seine Extremitäten. Morpholog. Jahrbuch, Bd. 25, pp. 465-543, 1897. Bd. 28, pp. 105-146, 1899.

——— Ueber eine Variation des kurzen Kopfes des Biceps Femoris beim Orang. Morpholog. Jahrbuch, Bd. 26, pp. 274-285, 1898.

Braus, H.—Die Entwickelung der Form der Extremitäten. Hertwig's Handbuch der Entwickelungslehre der Wirbelthiere, 1904.

Brooks, H. St. John.—Variation in the nerve supply of the lumbrical muscles in the hand and foot, with some observations on the perforating flexors. Journal of Anatomy and Physiology, Vol. 21, p. 575, 1887.

Bühler, A.—Morphologie des M. adductor magnus. Morpholog. Jahrbuch, Bd. 32, pp. 1-20, 1903.

Chudzinski.—Revue d' Anthropologie, 1874, p. 16.

Cunningham, J. D.—Journal of Anatomy and Physiology XII, XIII.

Eggeling, H.—Zur Morphologie der Damm-Muskulatur. Morpholog. Jahrbuch, Bd. 24, 1896.

Eisler, P.—Der Plexus lumbo-sacralis des Menschen. Halle, 1892.

——— Die Homologie der Extremitäten. Abhandl. naturforsh. Gesellschaft, Bd. XIX, Halle, 1895.

Ellenberger und Baum.—Anatomie des Hundes, 1891.

Forster, A.—Die Insertion des M. semimembranosus. Eine vergleichend-anatomische Betrachtung. Archiv für Anatomie und Physiologie. Anat. Abth., pp. 257-320, 1903.

——— Das Muskelsystem eines männlischen Papua. Neugeborenen. Abhandl. d. k. Leopold. Carol. Akad. d. Naturforscher, Vol. 82, p. 140.

Fürst, C. M.—Der Musculus popliteus und seine Sehne. Review by Bardeleben in Schwalbe's Jahresberichte, 1903, p. 230.

Frohse, E.—Ueber die Verzweigung der Nerven zu und in den menschlichen. Muskeln. Anatom. Anzeiger, Bd. 14, s. 321, 1898.

Gadow, H.—Beiträge zur Myologie der hinteren Extremität der Reptilien Morphol. Jahrb., VII, 1882.

Gegenbauer, C.—Vergleichende Anatomie der Wirbelthiere, 1898.

——— Lehrbuch der Anatomie des Menschen, 7 Aufl., 1898.

Gräfenberg, E.—Die Entwickelung der menschlichen Beckenmuskulatur. Anat. Hefte 72, pp. 429-494, 1904.

Gregor, A.—Ueber die Verteilung der Muskelspindeln in der Muskulatur des menschlichen Fœtus. Archiv. f. Anatomie und Physiologie, Anat. Abt. 1904, p. 112.

Grosser, O., and Froelich, A.—Beiträge zur Kenntniss der Dermatome der menschlichen Rumpfhaut. Morpholog. Jahrbuch, Bd. 30, pp. 508-537, 1902.

Gruber, W.—Anatomische Notizen. Virchow's Archiv, Bd. 103, 1886.

——— Extensor digiti II pedis longus.—Varietäten des M. extensor hallucis longus. Reichert und Du Bois Reymond's Archiv, p. 23, 1875.

Haach.—Vergl. Untersuchungen über die Muskulatur der Gliedmassen und des Stammes bei der Katze, dem Hasen, und dem Kaninchen. Berlin, 1904.

Halbertsma, H. J.—Ueber einen in der membrana interossea des Unterschenkels verlaufenden Nerven. Müller's Archiv, 1847.

Harrison, R. G.—An experimental study of the relation of the nervous system to the developing musculature in the embryo of the frog. This Journal, Vol. 3, pp. 197-220, 1904.

Henle, J.—Handbuch der systematischen Anatomie des Menschen. Braunschweig, 1871.

Holl, M.—Zur Homologie und Phylogenie der Muskeln des Beckenausganges des Menschen. Anatom. Anzeiger, Bd. 12, 1896.

Keith, A.—Notes on a theory to account for the various arrangements of the flexor profundus digitorum in the hand and foot of primates. Journal of Anatomy and Physiology, Vol. 28, 1894.

Klaatsch, H.—Der kurge Kopf des M. biceps femoris. Morpholog. Jahrbuch, Bd. 29, 217-281, 1902.

Kohlbrugge.—Muskeln und periph. Nerven der Primaten. Verhandl. der Akad. van Wetenschappen, Amsterdam, 1897.

Lannegrâce.—Myologie comp. des membres. Thèse, Montpellier, 1878 (cited by Le Double).

Leche, W.—Bonn's Thierreich, Bd. VI, Pt. V¹.

Le Double, A.—Traité des variations du système musculaire de l'homme. Paris, 1897.

Lewis, W. H.—The development of the arm in man. This Journal, Vol. 1, pp. 145-184, 1901.

Lubsen, J.—Zur Morphologie des ilium bei Saügern. Overdruk mit Petrus Campers, II, Abt. 3.

Nussbaum, M.—Nerve und Muskeln. Verhandl. d. anatom. Gesellschaft, 1894.

Pardi, F.—La morphologie comparata dei muscoli psoas minor, ilio psoas, e quadratus lumborum. Atti. d. Soc. Toscana di Scien. Natur., Pisa, 1902.

Parsons, F. G.—Expansion of tendons of gracilis and semitendinosus. Proceedings Anatom. Society. Journal of Anatomy and Physiologie, Vol. 38, p. 133, 1904.

Paterson, A. M.—The pectineus muscle and its nerve supply. Journal of Anatomy and Physiology, Vol. 26, 1891, and Vol. 28, 1895.

——— The origin and distribution of the nerves to the lower limb. Journal of Anatomy and Physiology, Vol. 38, pp. 84 and 169, 1894.

Porrier, P., and Charpy, A.—Traité d'anatomie humaine, T. 2, Paris, 1901.

Popowsky.—Zur Entwickelungsgeschichte der Dammmuskulatur beim Menschen. Anat. Hefte, Vol. 12, 1899.

Quain, I.—Elements of Anatomy, 10th ed., Vol. 2, Pt. 2, edited by G. D. Thane, London, 1892.

Ribbing.—Anat. Anzeiger, Bd. 28, p. 355, 1906.

Ruge, G.—Untersuchungen über die Extensorengruppe am Unterschenkel und Fuss der Saügethiere. Morpholog. Jahrbuch, Bd. IV, Suppl., 1878.

——— Zur vergleich. Anatomie der tiefen Muskeln der Fusssohle. Morpholog. Jahrbuch, Bd. 4, 1878.

——— Entwickelungsvorgänge an der Muskulatur des menschl. Fusses. Morpholog. Jahrbuch, Bd. 4, Suppl., 1878.

Salvi, G.—La filogenese ed i resti nell uomo dei moscoli pronatori personæotibiales. Monitore zoölg. italiano, Anno. 11, 1905.

Schomburg, Hans.—Entwickelung der Muskeln und Knochen des menschlichen Fusses. Gekrönte Preisschrift, Göttingen, 1900.

Schwalbe, G.—Ueber das Gesetz des Nervemuskeleintritts. Archiv Anatomie und Physiologie, Anat. Abth., pp. 167-174, 1879.

Sévéréano, G.—Du plexus lombaire. Bibliot. Anat., T. 13, 1905.

Taylor, G., and Bonney, V.—On the homology and morphology of the popliteus muscle. Journal of Anatomy and Physiology, Vol. 40, p. 34, 1905.

Tertut, L.—Les anamolies musculaires chez l'homme, expliquées par l'anatomie comparée. Paris, 1884.

Valenti, G.—Sopra le prime fasi di sviluppo della muscolatura degli arti. Archivo Ital. di Anatomie e Embryologie, Vol. 2, pp. 272-280, 1903.

Williams, Roger.—Journal of Anatomy and Physiology, Vol. 13, p. 204, 1878-9.

Wilson, J. F.—Abnormal distribution of nerve to quadratus femoris in man. Journal of Anatomy and Physiology, Vol. 23, p. 354, 1889.

Windle, B. C. A., and Parsons, F. G.—Morphology of the femoral head of the biceps flexor cruris. Journal of Anatomy and Physiology, Vol. 34, 1900.

DESCRIPTION OF PLATES.

Plate I.

Two figures, repeated from this journal (Vol. I, Plate II), to illustrate early stages in the differentiation of the inferior extremity.

Fig. 1. Embryo II, length 7 mm., age about four weeks.

Fig. 2. Embryo CLXIII, length 9 mm., age about four and a half weeks.

For description of figures see text, p. 264.

Plate II.

Four sections through the base of the posterior limb to illustrate different stages in the development of the nerves and musculature.

Fig. 1. Section passing through the right limb-bud in embryo II, length 7 mm., age 26 days. The tips of the neighboring myotomes do not extend into the mass of tissue of which the limb-buds are composed and in which as yet no specific differentiation is visible. 33 diam.

Fig. 2. Secton passing transversely through the base of the right limb-bud of embryo CIX, length 11 mm., age about five weeks. At the center of the limb-bud the acetabular region of the skeleton appears as a condensed mass of tissue. About this the femoral, obturator, and sciatic nerves may be seen extending into the limb bud. Myogenous tissue is fairly well marked near the femoral and sciatic nerves. 25 diam.

Fig. 3. Transverse section passing through the acetabular region of left leg of embryo CXLIV, length 14 mm., age about five and one-half weeks. The femoral, obturator, gluteal, and sciatic nerves may be seen extending into the limb bud, and in the vicinity of these nerves the anlages of the iliopsoas, pectineus, adductor, hamstring, and gluteal muscles. 25 diam.

Fig. 4. Transverse section passing through the acetabular region of embryo CXLV, length 33 mm., age about two months. The femoral, obturator, inferior gluteal, and sciatic nerves may be seen entering the limb. The chief fasciculi of the iliopsoas, pectineus, adductor, and gluteus maximus muscles are separated by an amount of connective tissue relatively greater than in the adult. 10 diam.

Plate III.

Three figures to illustrate the skeletal, muscular, and nervous apparatus of the right posterior extremity of embryo CIX, length 11 mm., age about five weeks. The nerves are represented black; the muscle anlages by stippling; the skeletal structures, light grey; the skin of the leg, transparent. About 17 diam.

Fig. 1. Median view. The urachus is shown in outline.

Fig. 2. Lateral view.

Fig. 3. Ventral view showing the relation of the pelvis to the body wall and the main nerve trunks. At the right the outline of the peritoneal membrane is shown. The division of the main nerve trunks into branches is diagrammatic.

Plate IV.

Medial and lateral views showing the relations of the nerves of the abdominal wall and posterior limb to the abdominal musculature and the skeleton of embryo CXLIV, length 14 mm., age about five and one-half weeks. The skin of the thigh and leg is represented transparent. About 17 diam.

Fig. 1. Medial view.

Fig. 2. Lateral view.

Plate V.

Medial and lateral views showing the relations of the nerves of the abdominal wall and posterior limb to the abdominal musculature and the skeleton of embryo XXII, length 20 mm., age about seven weeks. The skin of the leg is represented transparent. About 13 diam.

Fig. 1. Medial view.

Fig. 2. Lateral view.

Plate VI.

Figures showing the nerves of the abdomen and the nerves and muscles of the extensor side of the thigh.

Fig. 1. Embryo CXLIV, length 14 mm., age about five and one-half weeks. The abdominal musculature has been partially removed to show the course of the main nerve trunks. About 17 diam.

Fig. 2. Embryo XXII, length 20 mm., age about seven weeks. The ventral portion of the abdominal wall has been removed. About 13 diam.

Figs. *a* and *b*. Branches of the femoral nerve to the quadriceps femoris muscle in embryo CXLIV and in embryo XXII. The muscle in each instance is represented semitransparent. About 15 diam.

Plate VII.

Two diagrammatic outline sketches to illustrate the distribution of the cutaneous nerves of the inferior extremity in the adult.

Fig. 1. Front view of the left and medial view of the right leg. The dotted line on the right leg represents approximately the proximal margin of the embryonic limb-bud.

Fig. 2. Back of the left leg and lateral side of the right leg. The dotted line represents approximately the distal margin of the embryonic limb-bud.

Plate VIII.

Figures to illustrate the early differentiation of the adductor, hamstring, tensor fasciæ latæ, gluteal, obturator internus, and quadratus femoris muscles and the short head of the biceps and the nerves supplied to these. 25 diam.

Fig. 1. Adductor and hamstring muscles in embryo CXLIV, length 14 mm., age about five and a half weeks. In order that the adductor longus and brevis may be seen, merely the outline of the gracilis muscle is shown.

Fig. 2. Adductor group in embryo XXII, length 20 mm., age about seven weeks. The adductor brevis and the gracilis muscles are shown cut out a short distance from their attachments. In "a" the relation of the nerve to the belly of the gracilis muscle is shown; in "b" that to the belly of the adductor brevis. The muscles are represented as semitransparent so that the intramuscular course of the main nerve trunks may be followed.

Fig. 3. The hamstring group in embryo XXII. The belly of the semitendinosus has been cut out so as to show the deeper structures.

Fig. 4. The gluteal and obturator internus muscle groups in embryo CXLIV. The gluteus maximus muscle, except at its attachment to the femur, is represented merely by an outline. The central portion of the belly of the gluteus medius has been cut out to reveal the gluteus minimus. The tibial nerve is cut off near the plexus, the peroneal nerve more distally.

Fig. 5. The gluteal and obturator internus muscle groups in embryo XXII. The m. gluteus maximus and the lig. sacrotuberosum are shown in outline. The central part of the belly of the gluteus medius is cut out. The tibial nerve is cut off near the plexus.

Plate IX.

Six figures to illustrate the early differentiation of the musculature of the crus and pes. 25 diam.

Fig. 1. Peroneal and extensor muscles and nerves of the crus and pes of embryo CXLIV, length 14 mm., age about five and a half weeks. The peroneal muscles and the m. extensor digitorum longus are made semitransparent in order to reveal the deeper muscles and nerves.

Fig. 2. Peroneal and extensor muscles and nerves of the crus and pes of embryo XXII, length 20 mm., age about seven weeks. The peroneal muscles and the m. extensor digitorum longus are represented semitransparent.

Fig. 3. Superficial plantar musculature and nerves of the crus and pes of embryo CXLIV.

Fig. 4. Deep plantar musculature and nerves of embryo CXLIV. The gastrocnemius and flexor digitorum longus and the main trunk of the medial plantar and a part of that of the lateral plantar nerves have been removed.

Fig. 5. The superficial plantar muscles and nerves of the crus and pes of embryo XXII.

Fig. 6. The deep plantar muscles and nerves of the crus and pes of embryo XXII. The gastrocnemius and flexor digitorum longus muscles and the greater part of the tibial nerve have been removed.

Plate X.

Two figures to illustrate the early stages in the development of the pudic nerves and the distal portion of the sympathetic system.

Fig. 1. Medial view of the right half of the distal portion of embryo CXLIV, length 14 mm., age about five and a half weeks. Enough of the surrounding undifferentiated mesenchyme has been removed to reveal the course of the nerves and the neighboring blood-vessels. The intestines are not represented. 25 diam.

Fig. 2. Similar view of the right half of the distal portion of embryo XXII, length 20 mm., age about seven weeks. 20 diam.

ABBREVIATIONS USED IN LETTERING FIGURES.

abd. musc. ..... abdominal musculature.
acet. ..... acetabulum.
ao. ..... aorta.
A. ..... artery.
fem. ..... femoralis.
il. ext. ..... iliaca externa.
d. penis ..... dorsalis penis.
isch. ..... sciatic.
popl. ..... poplitea.
tib. ant. ..... tibialis anterior.
tib. post. ..... tibialis posterior.
umb. ..... umbilical.
calc. ..... calcaneus.
ch. d. ..... chorda dorsalis.
cœl. ..... cœlom.
costa ..... rib.
fe. ..... femur.
fi. ..... fibula.
ft. pl. ..... foot plate.
gl. suprar. ..... glandula suprarenalis.
il. ..... ilium.
isch. ..... ischium.
lig. sac. tub. ..... ligamentum sacrotuberosum.
lig. ing. ..... ligamentum inguinale.
meson. ..... mesonephros.
metan. ..... metanephros.
metat. ..... metatarsus.
M. ..... musculus (li).
abd. V ..... abductor digiti quinti
abd. hal. ..... abductor hallucis
add. ..... adductor group.
add. br. ..... adductor brevis.
add. hal. ..... adductor hallucis.
add. l. ..... adductor longus.
add. magn. ..... adductor magnus
add. min. ..... adductor minimus.
bi. ..... biceps femoris.
cap. br. ..... short head.
cap. l. ..... long head.
coccyg. ..... coccygeus.
cr. ant. ..... crurales anteriores.
cr. post. prof. ..... crurales posteriores profundi.
cr. post. supf. ..... crurales posteriores superficiales.
ext. dig. l. ..... extensor digitorum longus.
ext. dig. br. ..... extensor digitorum brevis.
ext. hal. l. ..... extensor hallucis longus.
ext. dig. V ..... extensor digiti quinti brevis.

fem. post.	hamstring group of muscles.
flex. dig. br.	flexor digitorum brevis.
flex. dig. V br.	flexor digiti quinti brevis.
flex. dig. l.	flexor digitorum longus.
flex. hal. br.	flexor hallucis brevis.
flex. hal. l.	flexor hallucis longus.
gastroc.	gastrocnemius.
gem.	gemelli.
gl. max.	gluteus maximus.
gl. med.	gluteus medius.
gl. min.	gluteus minimus.
gr.	gracilis.
il.	iliacus.
il. cost.	iliocostales.
il. ps.	iliopsoas.
interos. dors.	interossei dorsales.
interos. plant.	interossei plantares.
obl. abd. ext.	obliquus abdominis externus.
obl. abd. int.	obliquus abdominis internus.
obt. ext.	obturator externus.
obt. int.	obturator internus.
opp. V	opponens digiti quinti.
pect.	pectineus.
peron.	peroneal.
peron. br.	peroneus brevis.
peron. l.	peroneus longus.
pirif.	piriformis.
plant.	plantaris.
popl.	popliteus.
ps. mj.	psoas major.
quadr. fem.	quadratus femoris.
quadr. lumb.	quadratus lumborum.
quadr. pl.	quadratus plantæ.
quadri. fem.	quadriceps femoris.
r. abd.	rectus abdominis.
rect. fem.	rectus femoris.
sart.	sartorius.
semim.	semimembranosus.
semit.	semitendinosus.
sol.	soleus.
tens. fasc. lat.	tensor fasciæ latæ.
tib. ant.	tibialis anterior.
tib. post.	tibialis posterior.
trans. abd.	transversus abdominis.
vastus lat.	vastus lateralis.
vastus interm.	vastus intermedius.
vastus med.	vastus medialis.

myo. ..... myotome.
l. ..... lumbar.
s. ..... sacral.
t. ..... thoracic.
N.[32] ..... nervus (vi).
caud. ..... caudalis.
clun. inf. ..... clunium inferiores.
clun. med. ..... clunium mediales.
clun. sup. ..... clunium superiores.
cut. ant. ..... cutaneus femoris anterior.
dig. plant. ..... digitales plantares propr.
cut. lat. ..... cutaneus femoris lateralis.
cut. med. ..... cutaneus femoris anterior, medial branch.
cut. post. ..... cutaneus femoris posterior.
r. perin. ..... perineal branch.
cut. surœ lat. ..... cutaneus suræ lateralis.
cut. surœ med. ..... cutaneus suræ medialis.
d. penis ..... dorsalis penis.
fem. ..... femoralis.
g. ..... genital.
g. f. ..... genitofemoral.
gl. s. ..... gluteus superior.
gl. i. ..... gluteus inferior.
hœmorrh. med. ..... hæmorrhoidalis medialis.
hœmorrh. inf. ..... hæmorrhoidalis inferior.
hypog. ..... hypogastricus.
il. ..... iliacus.
ing. ..... inguinalis.
isch. ..... ischiadicus (sciatic).
l. ..... lumbalis.
l. ing. ..... lumbo-inguinalis.
obt. ..... obturatorius.
perin. ..... perinei.
peron. ..... peroneus.
peron. sup. ..... peroneus superficialis.
peron. prof. ..... peroneus profundus.
plant. ..... plantaris.
plant. l. ..... plantaris lateralis.
plant. m. ..... plantaris medialis.
pl. symp. ..... sympathetic plexus.
pud. ..... pudendus.
R. dors. l. ..... lateral cutaneus branch of the dorsal (posterior) division of a spinal nerve.
R. dors. m. ..... medial cutaneus branch.
s. ..... sacralis.

[32] For abbreviations of terms applied to nerves going to muscles, see under muscle, *M.*

saph. ..... saphenus.
sp. ..... spinalis.
sural. ..... suralis.
t. ..... thoracicus.
tib. ..... tibialis.
tr. symp. ..... truncus sympathicus.
visc. ..... visceral.
pat. .....patella.
perit. .....peritoneum.
proc. vag. .....processus vaginalis.
pub. .....pubis.
sp. ant. sup. .....spina anterior superior.
sp. post. sup. .....spina posterior superior.
symph. .....sympathetic nervous system.
tars. .....tarsus.
T. .....tendon.
test. .....testicle.
ti. .....tibia.
ur. .....ureter.
urach. .....urachus.
vert. .....vertebra.
V. .....vena.
card. ..... cardinalis.
d. penis ..... dorsalis penis.
fem. ..... femoralis.
hypog. ..... hypogastrica.
il. ext. ..... iliaca externa.
isch. ..... sciatic.
W. d. .....Wolffian duct.

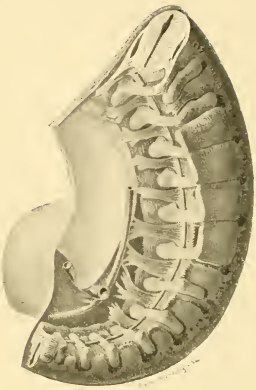


Fig. 1

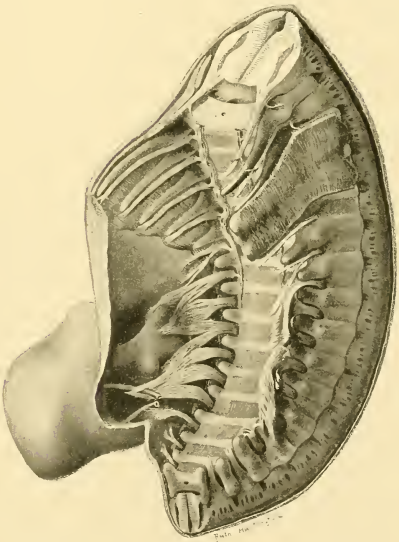


Fig. 2

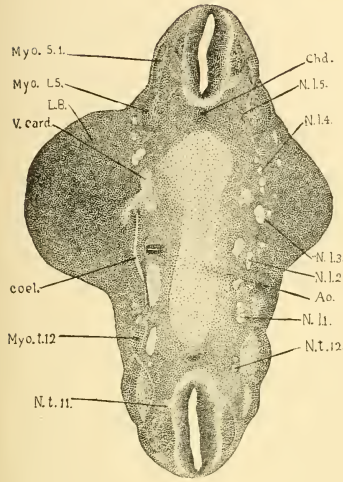


FIG. 1

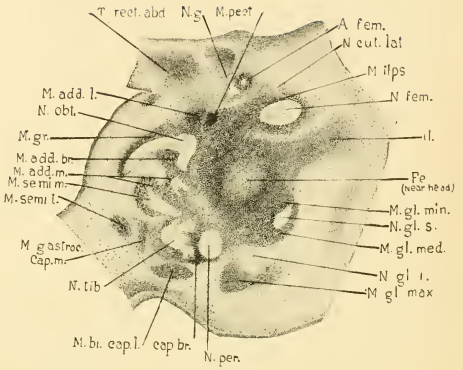


FIG 3

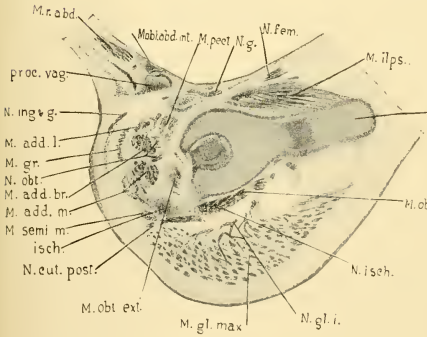


FIG. 4

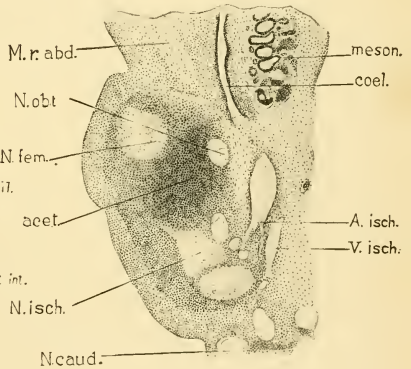


FIG 2

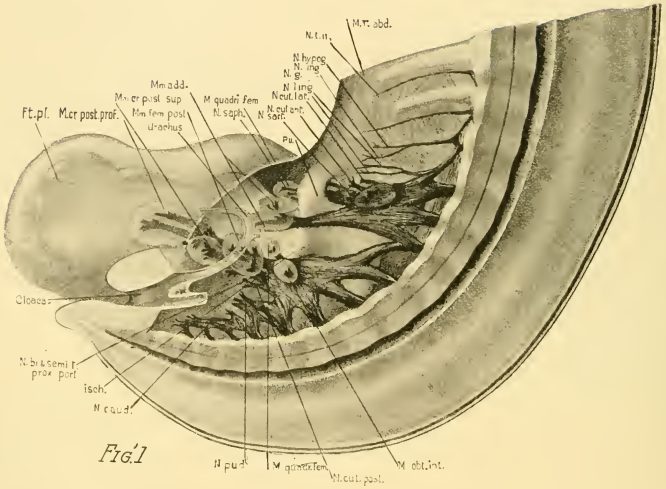

M.T. abd.
N.t.n.
N.hypog.
N. ing.
N. g.
N. ling.
Neut.lat.
N.cut.ant.
N.sart.
Pu.
Mm add.
M. cr post sup
Mm fem post
M quadri Fem
N. saph.
Ft.pf.
M.cr post prof.
Cloaca
N. br. semi t. prox. port
Isch.
N caud.
N pud.
M. quadr fem.
M. cut. post.
M. obt. int.

FIG. 1

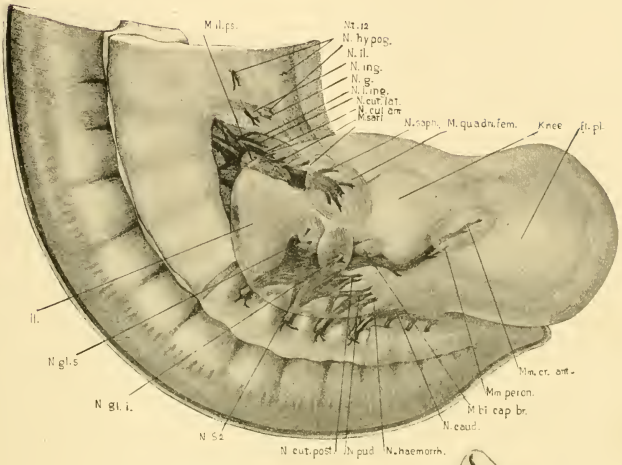

M. il. ps.
Nt. 12
N. hypog.
N. il.
N. ing.
N. g.
N. fem.
N. cut. lat.
N. cut. am
M. sart
N. saph.
M. quadr. fem.
Knee
ft. pl.
il.
N. gl. s
N. gl. i.
N. S2
N. cut. post.
N. pud
N. haemorrh.
N. caud.
M bi cap br.
Mm peron.
Mm. cr. ant.

FIG. 2

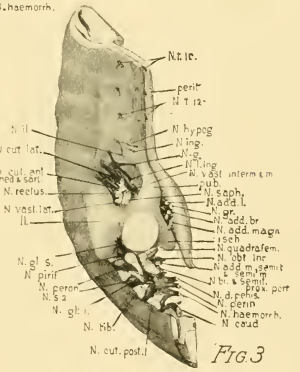

N.t. 12.
perit
N. il
N. hypog
N. ing.
N. g.
N. fem
N. vast. interm. & m. sub.
N. saph.
N. add. l.
N. gr.
N. add. br
N. add. magn
N. isch
N. quadr. fem.
N. obt. int
N. add. m. semit & semim
N. bi. & semit.
Pras. part
N. d. pelvis
N. perin
N. haemorrh.
N. caud
N. cut. post. f
N. gl. s.
N. pirif
N. peron
N. S 2
N. gl. i.
N. fib.

FIG. 3

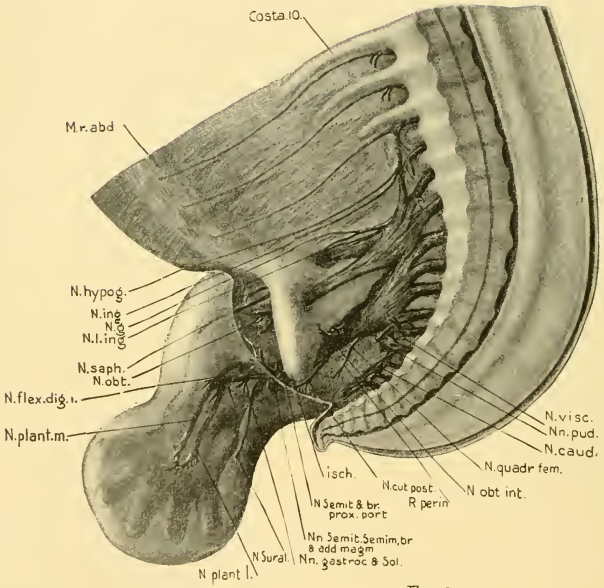

Costa 10.
M. r. abd
N. hypog.
N. ing
N. l. ing
N. saph.
N. obt.
N. flex. dig. 1.
N. plant. m.
N. visc.
Nn. pud.
N. caud.
N. quadr fem.
N. obt int.
N. cut post
R perin
Isch.
N Semit & br. prox. port
Nn. Semit. Semim. br & add magm
Nn. gastroc & Sol.
N Sural
N plant l.

FIG. 1

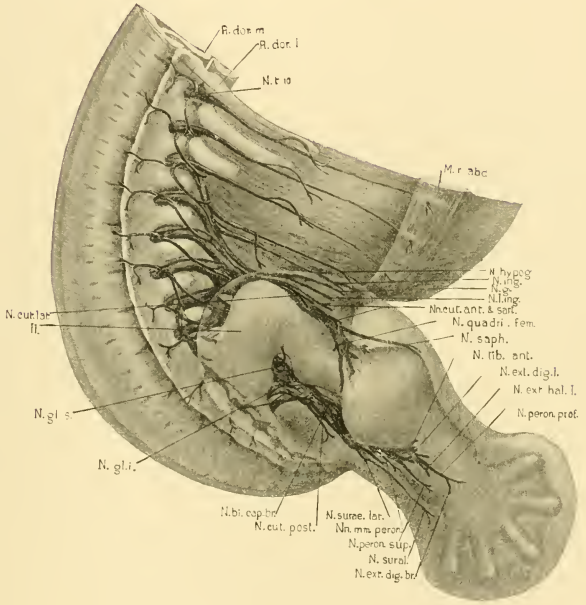

R. dor. m.
R. dor. l.
N. cut. lat. II.
N. gl. s.
N. gl. i.
N. hypog.
N. ing.
N. isch.
N. ing.
Nn. cut. ant. & sart.
N. quadri. fem.
N. saph.
N. tib. ant.
N. ext. dig. l.
N. ext. hal. l.
N. peron. prof.
N. bi. cap. br.
N. cut. post.
N. surae. lat.
Nn. mm. peron.
N. peron. sup.
N. sural.
N. ext. dig. br.

Fig. 2

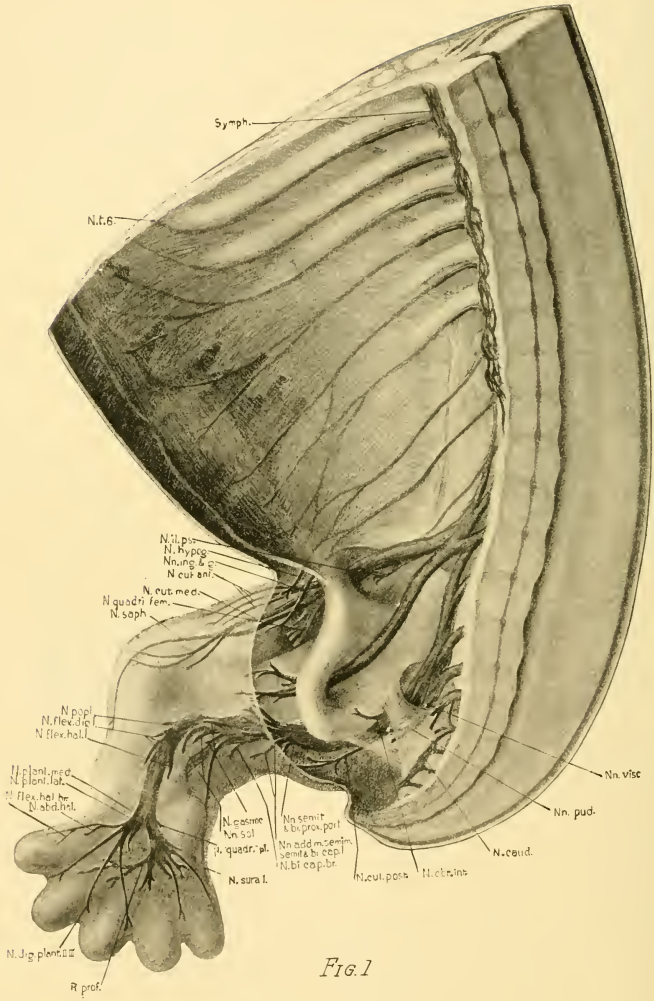

Symph.
N.f.6.
N.il.pso.
N. Hypog.
Nn.ing.& g.
N.cut.ant.
N. cut.med.
N.quadri fem.
N. saph
N.popl.
N.flex.dig.l.
N.flex.hal.l.
N.plant.med.
N.plant.lat.
N.flex.hal.br.
N.abd.hal.
N. gastroc. & sol.
N. quadr. pl.
N. sura l.
N.plant.prof.
R prof.
Nn.semit. & bi.pro.port.
Nn.add.m.semim. semit.& bi.cap.l.
N.bi cap.br.
N.cut.post.
N.ischi.inf.
N.caud.
Nn. pud.
Nn. visc.

FIG. 1

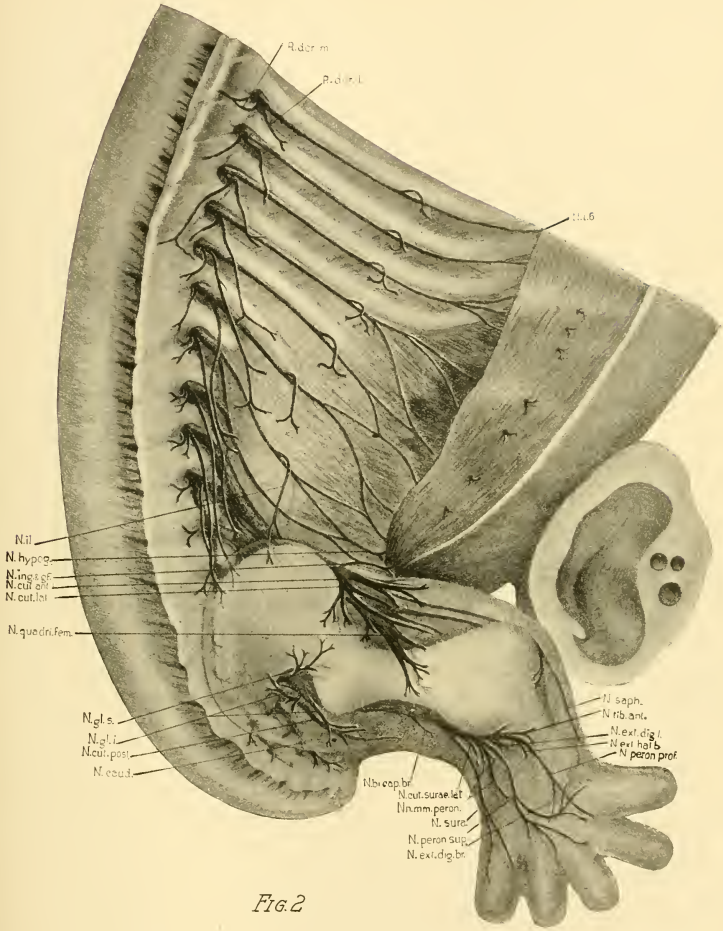

N.il
N. hypog.
N. ing. gf.
N. cut. ab.
N. cut. fem.
N. quadr. fem.
N. gl. s.
N. gl. i.
N. cut. post.
N. cut. f.
N. saph.
N. tib. ant.
N. ext. dig. l.
N. ext. hall.
N. peron prof.
N. biceps br.
N. cut. surae lat.
N. mm. peron.
N. surae
N. peron sup.
N. ext. dig. br.

Fig. 2

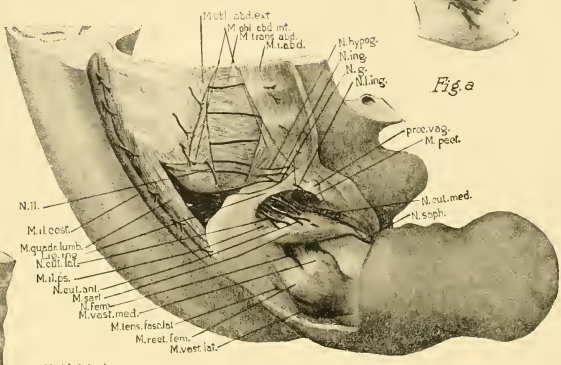


Fig. 1

Fig. a

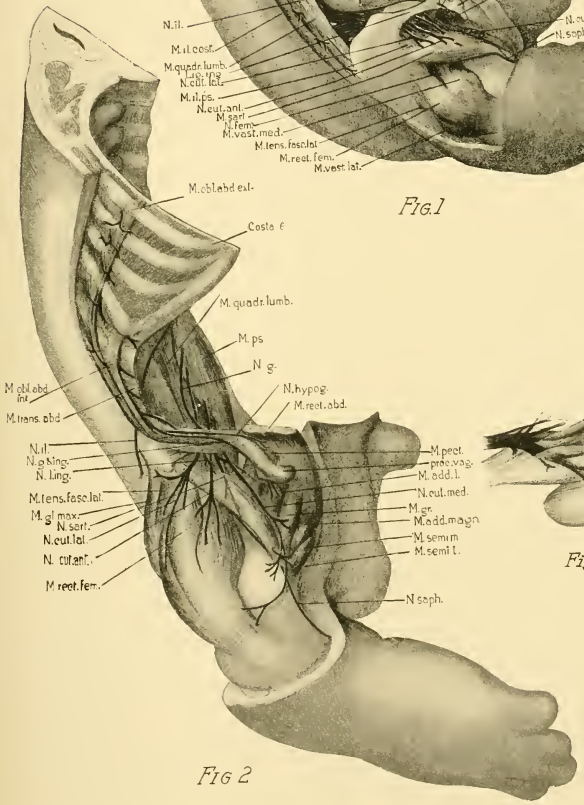


Fig 2

Fig. b.

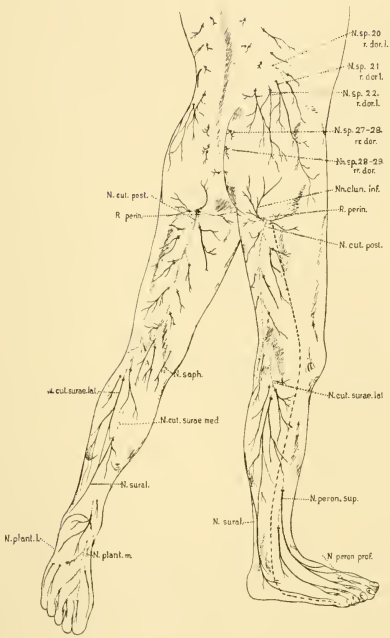

N. sp. 20 r. dor. l.
N. sp. 21 r. dor. l.
N. sp. 22 r. dor. l.
N. sp. 27-28 rr. dor.
N. sp. 28-29 rr. dor.
N. clun. inf.
R. perin.
N. cut. post.
N. cut. post.
R. perin.
N. cut. surae. lat.
N. saph.
N. cut. surae med.
N. sural.
N. peron. sup.
N. sural.
N. plant. l.
N. plant. m.
N. peron prof.

FIG. 2

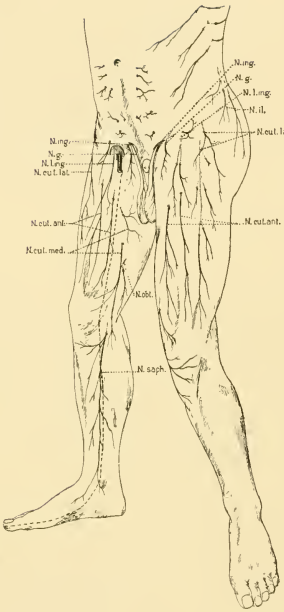

N. ing.
N. g.
N. l. ing.
N. il.
N. cut. lat.
N. ing.
N. g.
N. l. ing.
N. cut. lat.
N. cut. ant.
N. cut. ant.
N. cut. med.
N. obt.
N. saph.

FIG. 1

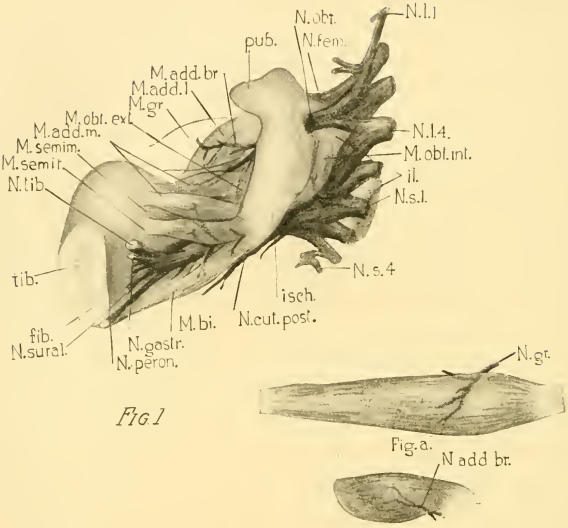

N.obt.
N.l.1
pub.
N.fem.
M.add.br
M.add.l
M.gr
M.obt.ext.
M.add.m.
N.l.4.
M.semim.
M.obt.int.
M.semit.
il.
N.tib.
N.s.l.
N.s.4
tib.
isch.
M.bi.
N.cut.post.
fib.
N.sural.
N.gastr.
N.peron.
N.gr.
Fig.a.
N add br.
Fig.b.

Fig 1

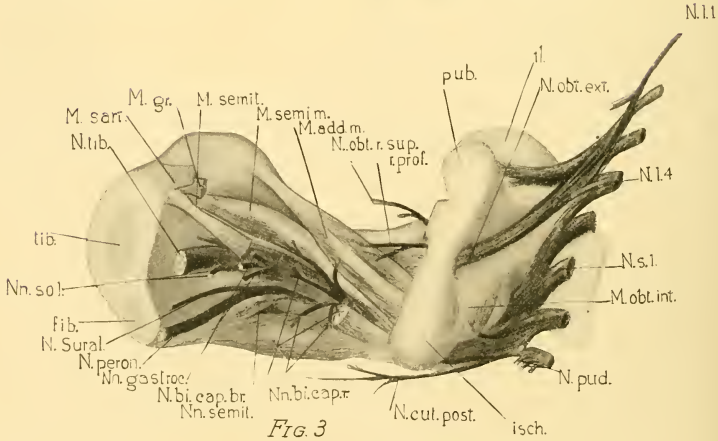

N.l.1
pub.
il.
N.obt.ext.
M. gr.
M. semit.
M. sart.
M. semim.
M.add m.
N.tib.
N..obt. r. sup.
r.prof.
N.l.4
tib.
N.s.1.
Nn. sol.
M.obt. int.
fib.
N. Sural
N. peron.
Nn. gastroc.
N. pud.
N.bi. cap. br.
Nn. bi. cap. r.
Nn. semit.
N.cut. post.
isch.

Fig. 3

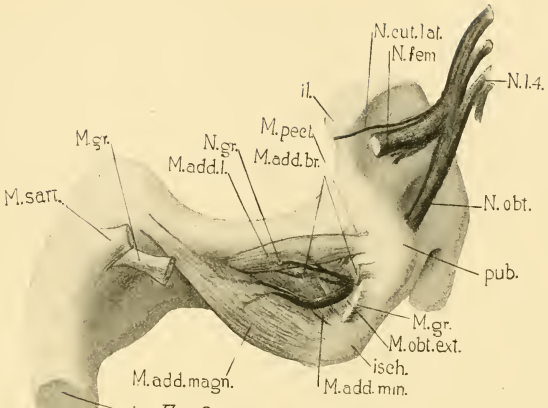


Fig 2

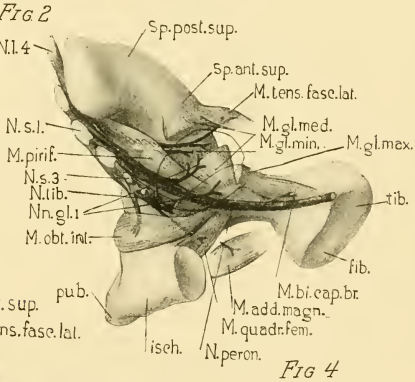


Fig 4

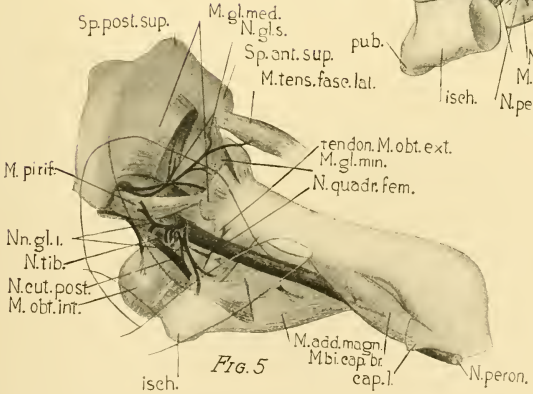


Fig. 5

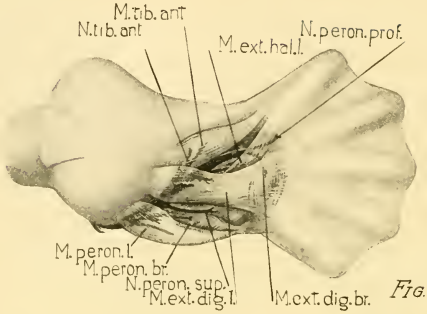


FIG. 1

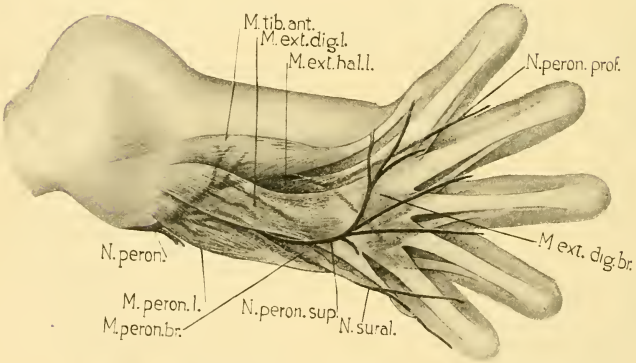


FIG. 2

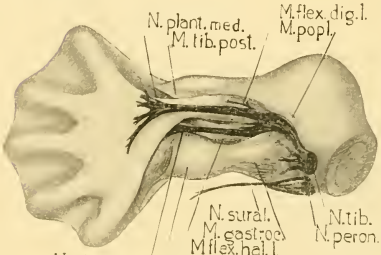


FIG. 3

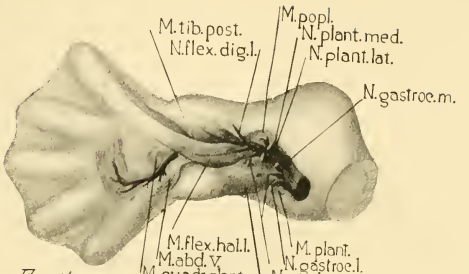


FIG. 4

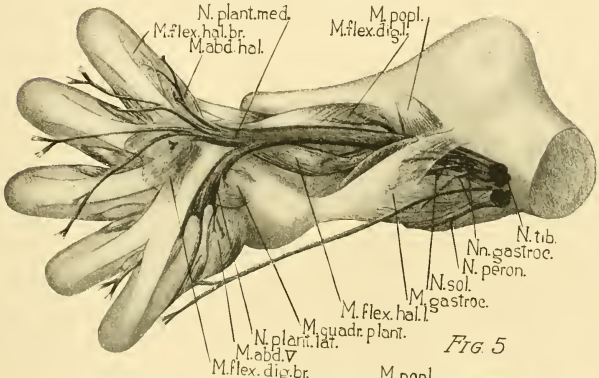


FIG. 5

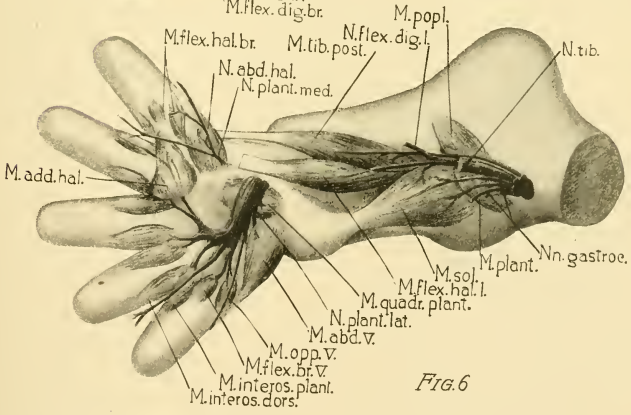


FIG. 6

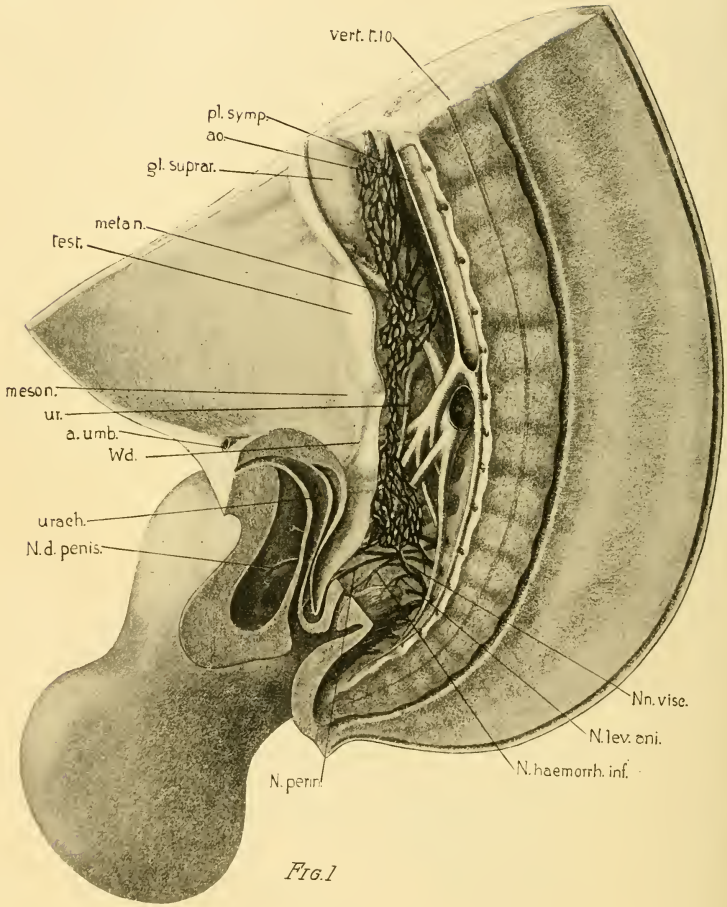

vert. r.10
pl. symp.
ao.
gl. suprar.
metan.
rest.
meson.
ur.
a. umb.
Wd.
urach.
N. d. penis.
Nn. vise.
N. lev. ani.
N. haemorrh. inf.
N. perin.

FIG. 1

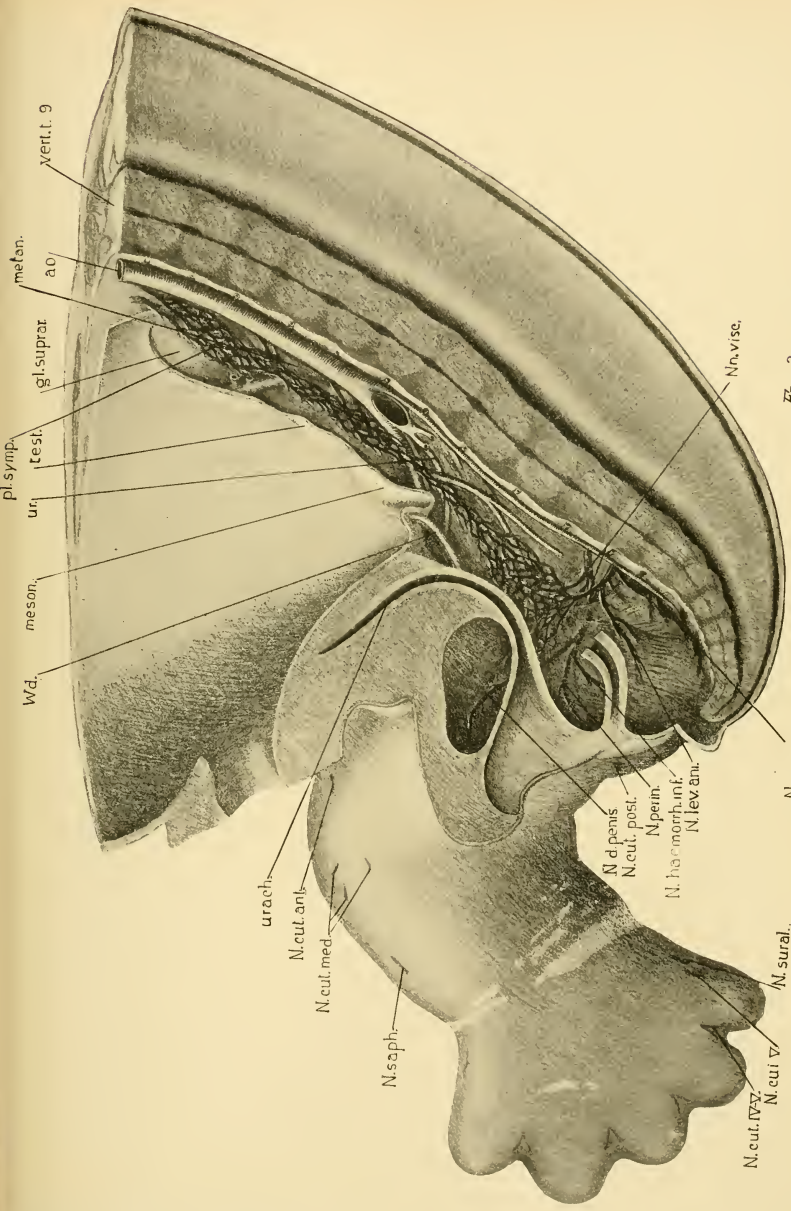

Vert. l. 9
metan.
ao.
gl. suprar.
pl. symp.
test.
ur.
meson.
Wd.
Nn. visc.
N. caud.
N. d. penis
N. cut. post.
N. pud.
N. haemorrh. inf.
N. lev. an.
N. cut. ant.
N. cut. med.
N. saph.
N. sural.
N. tib. V
N. per. V

Fig. 2

THE ARTERIOLÆ RECTÆ OF THE MAMMALIAN KIDNEY.[1]

BY

G. CARL HUBER,

From the Laboratory of Histology and Embryology of the University of Michigan.

WITH 4 TEXT FIGURES.

In a comprehensive and relatively recent contribution on the blood supply of the mammalian kidney, Golubew[1] calls attention to the differences of views still existing concerning the minute anatomy of this organ and states that of these controversial questions special mention may be made of the "vasa recta of Henle and Donders or of the arteriolæ rectæ of authors." A study of the literature which is fully reviewed by Golubew leads him to state that at the time of his communication three views were current pertaining to the origin of the arteriolæ rectæ. According to one view, these vessels arise from the vasa efferentia of the glomeruli which lie nearest to the pyramid of the kidney, a view early expressed by Bowman who was followed by Gerlach, Kölliker, and Ludwig in their earlier writings. According to another view, recognition and prominence are given to the arterial branches forming straight medullary vessels which arise directly from the renal vessels and their branches without the interposition of glomeruli and known as the arteriolæ rectæ veræ. According to a further view maintained by Huschke and other observers, the origin of the arteriolæ rectæ was traced to the capillary plexuses surrounding the tubules of the cortex of the kidney. Steinach, who denies the existence of arterial straight medullary branches, presents a view which cannot be included in the above classification and may be disregarded as his observations have not met with acceptance. Virchow and many other observers who have followed him have to some extent harmonized these conflicting views by assuming what may be regarded as a middle position in that they recognize the arteriolæ rectæ veræ, vessels

[1] Golubew: Ueber die Blutgefässe der Niere der Säugetiere und des Menschen. International monatsschr. f. Anat. u. Physiol., Bd. X, 1893. Gives references to literature appearing before the date of his publication.

which arise directly from the renal vessels, but concede the presence of straight medullary vessels which have their origin in the efferent branches of the glomeruli situated in the deeper layers of the cortex and known as the arteriolæ rectæ spuriæ. Golubew, whose very careful work has justly received merited consideration, describes and figures both arteriolæ rectæ veræ and spuriæ. A study of the diagrams of the renal circulation as found in the recent text-books of Anatomy and Histology warrants the conclusion that the majority of the present day writers believe in the double origin of the arteriolæ rectæ, namely in part directly from the renal vessels, for the remainder, from the efferent branches of glomeruli.

Of the various methods that have been used in the study of the arteriolæ rectæ, the injection methods in one form or another are given preference. Golubew used colored gelatin masses injected through either the renal artery or vein, but more particularly a solution of silver nitrate which was injected into the vessels after these had been thoroughly washed out with distilled water. The silver nitrate was injected under low pressure and the injection interrupted as soon as reduction of silver was evident in the capsule of the kidney, after which the vessels were again washed out with distilled water, the organ divided into pieces, placed in alcohol and exposed to light. Free hand sections, dehydrated and cleared in oil of cloves and mounted in damar were used for study. Corrosion preparations of the renal vessels obtained mainly with the celloidin method have enabled Brödel [2] and others who have confirmed him to extend our knowledge of the general distribution, relations, and manner of termination of the renal vessels. The results thus obtained have also been confirmed in Roentgen photographs taken after suitable injection of the renal vessels. Particular attention was, however, not given to the arteriolæ rectæ by these observers.

The observations here briefly to be recorded were made on a series of corrosion preparations of the renal vessels of the dog, cat, rabbit, rat, and guinea pig made after a method which is a modification of one suggested by Krassuskaja.[3]

This observer recommended an injection mass consisting of photoxyliu (or celloidin), camphor and acetone, colored by the addition of pigments rubbed up in acetone. (For a red color, cinnabar is suggested; for a blue, Berlin blue; a yellow, chrome yellow; a black, asphalt.) The mass may be

[2] Max Brödel: The Intrinsic Blood-Vessels of the Kidney and Their Significance in the Nephrotomy. Proc. Ass. Amer. Anat., 1901.

[3] A. Krassuskaja, as reviewed by Stieda in Ergeb. Anat. u. Entwickl., Bd. XIII, 1903, p. 521.

filtered through flannel or muslin. Injection may be made by means of an ordinary syringe; it is only necessary to fill the cannula with acetone. The tissue or organ is placed into hydrochloric acid 12 to 24 hours after the injection and is removed from the acid after two or three days and washed in flowing water. The pigments suggested by Krassuskaja are not soluble in acetone and are, therefore, only held in suspension. The mass did not seem to me suitable for injecting capillaries and other exceedingly fine tubular structures. This led to a modification of it and the mass now used in this laboratory is as follows:

To obtain a stock solution, 30 grms. of photoxylin are dissolved in 550 cc. of acetone, which requires about 24 hours. Twenty grms. of camphor are dissolved in 50 cc. of acetone. The two solutions are then thoroughly mixed

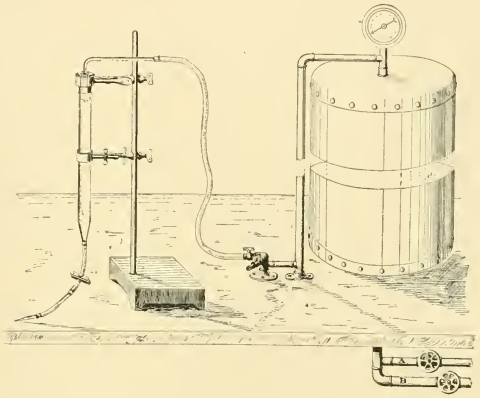


Fig. 1. Injection apparatus: *A*, pipe carrying water to tank; *B*, conveying water from tank and connected with waste-pipe. For further details see text (page 394).

in a bottle with a well-fitting glass stopper. This stock solution may be kept for a long time. After experimenting for a long time, it was found that Alkanin[4] answered very well for the purpose of a red color. It is readily soluble in acetone, is not washed out of the preparation with water, alcohol, or xylol, and is not decolored in the hydrochloric acid. It is the only substance soluble in acetone and meeting the other requirements which I have thus far been able to find. The preparation of alkanin first used was one that had been in the laboratory a long time and had become hard and brittle. The alkanin, as obtained from Gruebler, is in the form of a thick paste. Later observations have shown that the dried form answers the purpose better than a fresher preparation. The injection mass now used is made by

[4] Fettlösliches Roth, Gruebler, also written Alcanin.

adding 0.3 to 0.5 grms. of the alkanin rubbed up in 20 cc. of acetone to 80 cc. of the stock solution, thoroughly mixed by stirring in a mortar and then filtered through absorbent cotton with the aid of a Chapman suction pump. For purposes of injection, I have made use of compressed air, obtained by connecting a water tank with the laboratory water pipe. The tank is provided with a pressure gauge, from which the pressure obtained is read. The pressure is conveyed to the table by means of a gas-pipe, provided with a stop-cock. The injection mass is placed in a large glass tube with 3 cm. lumen and about 20 cm. long, held upright by clamping the same to a support. The upper end of the tube is provided with a perforated rubber cork, which can be clamped in tightly. A rubber tube leads from the end of the pipe bringing the compressed air to the table to a short glass tube fastened in the rubber cork, by means of which the pressure is conveyed to the injection mass. To the lower end of the glass tube, which tapers, is attached a rubber tube, provided with a clamp, by means of which connection may be made with the cannula. The simplicity of this apparatus commends itself. It is shown in Fig. 1. As is usual in injections, better results are obtained by injecting a limited area, that is directly through the blood-vessel supplying the organ to be studied. It has not been found necessary to wash out the blood-vessels before injecting. The animal is bled as freely as possible by severing the neck-vessels before death. The administration of amyl nitrite does not appear to influence materially the completeness of the injection. The injection is to be made soon after the death of the animal. The cannula is first filled with normal salt, and the salt solution renewed by means of a pipette until it remains clear in the cannula. It is then replaced with acetone, which is likewise renewed several times to dehydrate the interior of the cannula. If the area to be injected is very small, a portion of the acetone is withdrawn from the cannula and this is filled with the injection mass. Better results are obtained by using relatively high pressure. In injecting the renal arteries of the dog, cat, and rabbit, a pressure of 20 to 25 pounds, as registered by the gauge connected with the tank, gave the best injection; for smaller animals, 12 to 15 pounds. Better results are also obtained if the full pressure to be used is thrown onto the mass as quickly as possible. The pressure is maintained for five to ten minutes. Before removing the cannula from the vessel, the vessel should be tied distal to the cannula, so as to avoid a back flow. A 75 per cent solution of hydrochloric acid (sp. gr. 1.20) is used for macerating the parts to be removed. The entire organ may be placed in this macerating fluid, or, as it is often desirable to study a corrosion in small pieces, the organ may be cut into segments as desired and these placed into the macerating fluid. The injected tissue may be placed into the macerating fluid 10 to 20 minutes after the completion of the injection; there is no advantage in waiting 12 to 24 hours, as recommended by Krassuskaja. Pieces with one diameter not more than 1 cm. are thoroughly macerated in 18 to 24 hours. The macerated pieces are then transferred to a large dish of water and the softened tissues removed by playing water against them with a dropper provided with a rubber bulb. It is not advisable to use a stream of water with considerable force, as the delicate parts of the corrosion are likely to be injured. After the corrosion has been thoroughly cleaned, it may be allowed

to dry or it may be studied in water. I have found it advantageous to mount in balsam the parts to be studied particularly. If this is desired, the thoroughly cleansed corrosions are placed in distilled water for several hours, are then dehydrated in absolute alcohol, transferred to xylol and mounted in balsam, the cover glass being supported by fragments of glass of the required thickness. These preparations should be viewed with a binocular microscope giving stereoscopic vision.

This method has proven very satisfactory in the study of the renal vessels, as it has often been possible to obtain corrosions in which the course of the vessels could be readily followed through their several divisions until the capillaries are reached. In such preparations, a confusion of arterial and venous branches is not possible. The method enables a definite solution of the course and divisions of the major branches of the renal artery, of the radiate cortical branches (arteriæ interlobulares), of the origin of the afferent branches to the glomeruli and of the fate of the efferent glomerular branches. It is the purpose at this time to consider primarily the arteriolæ rectæ and other efferent glomerular branches; a fuller consideration of the renal vessels, both arterial and venous, is reserved for further contribution.

As is well known, the renal artery, on entering the hilus of the kidney, divides into branches which, after division, course in the peripheral part of the pyramid near the junction of the medullary and cortical portions. (This statement has reference to the kidneys particularly studied, namely, those with a single pyramid.) These major branches, which in their course undergo several subdivisions, have a direction which is in the main parallel to the surface of the kidney; they describe, therefore, arcs with convexity outward, and constitute the arterial branches designated as arcuate arteries (arteriæ arciformes). From the convex side of these arcuate arteries, there arise at intervals of 2 to 5 mm. branches which very generally form an acute angle with the arcuate artery and pass with slight inclination toward the cortex. The length of these branches varies, and from their outer side, that toward the cortex, there arise short branches at relatively close intervals which pass toward the periphery of the kidney and very generally subdivide into several branches from which arise the so-called interlobular arteries (arteriæ interlobulares). The arcuate arteries ultimately terminate in smaller branches which also give origin to interlobular arteries. Golubew simply states that from the convex side of these arches (arteriæ arcuatæ), as also from the terminal divisions, arise the interlobular arteries. Von Ebner[5] after mentioning

[5] In Kœlliker's Handbuch, Vol. III, Pt. 1, page 369.

the arteriæ arciformes. states that " from the cortical side of these there arise with great regularity and mostly at right angles small arteries which, after several or more repeated divisions, end in fine branches of 135 to 220 μ caliber, which, with a straight course, pass outward between the cortical fasciculi (Rindenfascikeln) or lobules and are most appropriately termed the arteriæ interlobulares." If the designation arteriæ arciformes is retained for arterial branches having an arched course, relatively large branches arising from these and passing through two to three further subdivisions need to be recognized before arterial branches known as the arteriæ interlobulares are reached. The usual description of the interlobular arteries is also open to question. Arterial branches passing quite regularly with radial course through the cortex as generally diagrammed are seldom met with. This must be evident to one who has had opportunity to observe numerous kidney sections of material injected with a colored gelatine mass, as usually given to classes, and to note the relative infrequency with which sections are met showing interlobular arteries which may be traced from the deeper portion of the cortex to the periphery. Branching of the interlobular vessels at various levels of their course is frequently met with; certain ones pass only through a portion of the cortex, others again break up in the deeper portions of the cortex into clusters of smaller branches (afferent glomerular branches). These details are shown in the figures presented. If the interlobular arteries are to be regarded as associated with vascular units, it must be conceded that such units must vary greatly in shape and in relative position and recognition must be given to the fact that of the probable functional activity of each uriniferous tubule structurally associated with an interlobular artery, only a portion of this functional activity is associated with the portion of the uriniferous tubule which falls within the vascular area of an interlobular artery, as the loops of Henle of such tubules are generally situated outside of such a vascular area.

It seemed desirable to discuss thus briefly certain points in the arterial vascular system of the mammalian kidney in order that emphasis may be given to the statement that afferent glomerular branches arise from all the branches of the renal artery, beginning with the arteriæ arciformes, and that all the branches with the few exceptions to be mentioned terminate in glomeruli. The main exceptions are found in the A. nutriciæ pelvis renalis and within the sinus renalis the arteriæ recurrentes. These branches, especially the latter, are readily recognized in corrosions, although their relations to the structures which they supply are not evident in such preparations. The arrangement of their terminal

branches is, however, such that they are not to be confused with the arteriolæ rectæ. They generally arise from primary branches of the renal arteries. Other exceptions will be noted later. In corrosions of very fully injected material, there are often observed small branches arising generally from the concave side of the arcuate arteries, beginning with about the third division of these, which can be traced to glomeruli; they are, therefore, afferent glomerular vessels. These small branches are not numerous. Their length varies from 1 mm. to much less than that. They generally end in only one glomerulus, though now and then such an afferent glomerular branch divides to supply two glomeruli. On the branches which arise from the convex side of the arcuate arteries and through their second and third divisions, afferent glomerular branches become more numerous, the number increasing with each successive division of these arterial branches. These afferent glomerular branches generally arise from the under surface (toward medulla) or the sides of these larger arterial stems, though now and then from the upper surface, in which event the branch bends downward to reach the respective glomerulus. Such afferent glomerular branches vary in length and arrangement. Branches ending in a single glomerulus are met with; clusters of two, three, four, or even more afferent branches, each ending in a glomerulus are also seen. Numerous afferent glomerular branches arise from the arterial branches which divide to form the interlobular arteries. Here also they may arise singly or in small groups or a small arterial twig may divide into four, six to eight branches, each ending in a glomerulus. From the interlobular arteries, as is generally stated, arise at all levels through the cortex and from all sides numerous afferent glomerular branches. Attention may, however, be drawn to the fact that the arrangement of afferent glomerular branches arising from the interlobular arteries is not a regular one, single afferent branches or clusters consisting of two to five or even more such branches resulting from a division of small lateral twigs of the interlobular arteries are met with. The terminal portions of such interlobular branches as reach the periphery of the cortex, ultimately divide into afferent glomerular branches. The number of such terminal afferent glomerular branches thus formed in the periphery of the cortex varies with different interlobular arteries. The figures presented will serve to elucidate this statement. It should, however, be stated that Figs. 2, 3, and 4 are drawn from actual preparations and are not composite pictures, and it will be readily understood that not all of the preparations present all of the details with equal clearness and perfection. Even when care is taken to cut and tease out certain portions

of a corrosion, selected for mounting and special study, which may readily be done under the binocular stereoscopic microscope, there are broken off during the manipulation small portions which thus become detached from the preparation. In Figs. 3 and 4, for instance, the peripheral portions of the interlobular arteries are not in every instance fully injected, giving the impression that certain of the interlobular arteries present peripheral branches which do not end in glomeruli. Other corrosions in which the peripheral branches of the interlobular arteries were more fully injected, but in which the injection in other details was not wholly successful will serve to show that the interlobular arteries end at the periphery of the cortex in branches which are recognized as afferent glomerular branches.

As is well understood, each glomerulus constitutes a rete mirabile, its branches uniting to form a single efferent vessel, the vas efferens, which is regarded as an arterial and not a venous structure. The efferent glomerular vessels, soon after leaving the glomeruli, divide to form capillaries, the disposition of which differs in the different portions of the kidney. The efferent branches of the glomeruli, the afferent branches of which arise from the arcuate arteries and from the successive branches of these until the interlobular arteries are reached, as also the efferent branches of a varying number of the glomeruli the afferent branches of which spring from the lowermost portions of the interlobular arteries, divide into bundles of long, slender branches and capillaries which pass into the medulla of the kidney, constituting the arteriolæ rectæ of writers, more specifically stated the arteriolæ rectæ spuriæ. The efferent branches of the remaining glomeruli divide to form capillary plexuses which surround the segments of the renal tubules found in the cortex, the efferent branches of the glomeruli situated in the outermost portion of the cortex passing into the peripheral cortical region free from glomeruli, before forming capillary plexuses. It may here be emphasized that there is not a difference of kind in the capillary plexuses formed from the efferent glomerular branches in the different parts of the kidney, but one of plexus arrangement determined by the character and arrangement of the tubular structures found in the different regions of the kidney.

As has been previously stated, the majority of recent writers recognize the existence of terminal arterial branches which end in capillaries in the kidney substance, with which glomeruli are not associated, such branches conveying arterial blood to the kidney tubule or portions thereof, which has not passed through a glomerulus. Such branches are recog-

nized in the boundary zone and medulla as arteriolæ rectæ veræ and in the peripheral portion of the cortex as end branches of the interlobular arteries. The criticism may be made that the corrosion method employed is not suitable for determining the existence or non-existence of such branches, as the possibility of their being present without being injected must be considered. It would seem, however, reasonable to suppose that arteriolæ rectæ veræ should be more readily injected than arteriolæ rectæ spuriæ, since in injecting the former it would not be necessary for the injection mass to pass through the glomerular capillaries before reaching the branches and capillaries constituting the arteriolæ rectæ. The venæ rectæ are very readily injected through the veins. In the rat, guinea pig, and rabbit and practically without exception in the cat, the arteriolæ rectæ observed in my corrosions could readily be traced to the efferent glomerular vessels. In these forms then, the existence of arteriolæ rectæ veræ may be denied with the possibility of very rare exceptions in the cat. A similar conclusion is reached by Petraroja, whose account I have, however, seen only in review, as his original publication was inaccessible to me.[6] In the dog, I have now and then observed arterial twigs which terminate directly in arteriolæ rectæ—arteriolæ rectæ veræ—these constitute, however, a very small per cent, the great majority resulting from a division of efferent glomerular branches. In the dog there may be further observed what may be designated as very small glomeruli, which appear fully injected as a capillary network may generally be made out in the corrosion, the efferent branch ending in typical arteriolæ rectæ. These very small glomeruli (?) are also not numerous. Golubew has described and figured for the dog and the cat what he has termed "retia mirabilia renum nova" situated in the deeper portion of the cortical substance and the boundary zone. Being aware of these observations of Golubew, I sought for confirmation of them in the corrosion preparations at my disposal, as it seemed likely that they should be injected as readily as the glomerular vessels. Such retia mirabilia have not been found, unless, as seems to me probable, what has been spoken of as very small glomeruli may constitute the structure described by Golubew as new renal retia mirabilia. From the fact that the efferent vessels of such structures always end, so far as I have been able to determine, in arteriolæ rectæ, I have been led to conclude that they represent the remains of normal glomeruli associated in their development with urinifer-

[6] Petraroja: Sulle arteriolæ rectæ del rene. Monit. Zool. ital., Bd. 15, 1904. Reviewed in Jahresberichte über die Fortschritte der Anatomie und Entwickelungsgeschichte. Neue Folge, Bd. X, 3 Abth. 1. Teil, 1905.

ous tubules, which tubules have later disappeared, a portion of the glomerular plexus with afferent and efferent vessels remaining intact as a rete mirabile, the degree of retrogressive change varying with different glomeruli and in some instances going on to a complete obliteration of the glomerular plexus, the afferent and efferent vessels alone remaining as a continuous structure. As has been expressed to me—"The mill-dam remaining after the mill has disappeared and in some few instances the dam itself disappearing." According to this hypothesis (for the sub-

FIG. 2. Corrosion preparation of terminal arterial branches from kidney of dog.

stantiation of which it is difficult to obtain definite data, as my attempts to obtain corrosion preparations of the arterial system of fœtal kidneys and of kidneys of new-born dogs have not been successful), the arterial twigs which end in arteriolæ rectæ without the interposition of glomeruli —arteriolæ rectæ veræ—and the arterial twigs in the course of which are found retia mirabilia prior to ending in arteriolæ rectæ are to be regarded as derived from glomeruli with afferent and efferent vessels, the glomerulus in each instance degenerating in whole or in part consequent to the disappearance of the uriniferous tubule with which said glomerulus

was structurally associated. If this interpretation be correct, the arterial vascular supply of the dog forms only an apparent exception to the general statement that all the arteriolæ rectæ are formed by a division of efferent glomerular branches, the few arteriolæ rectæ veræ noted being regarded as developed from arteriolæ rectæ spuriæ on the disappearance of the uriniferous tubule structurally associated with the glomerulus which thus degenerates. Golubew has further described arterial twigs, which after division present one branch, which may be very short, and which forms an afferent glomerular vessel, the other branch passing by but in close contiguity to the glomerulus and dividing to form arteriolæ

Fig. 3. Corrosion preparation of terminal arterial branches from kidney of cat.

rectæ—thus arteriolæ rectæ veræ. This I have not observed and must regard it as an error of observation for which the method used by him (silver nitrate injection) is responsible, as is clearly the case in the following observation, shown in his Fig. 2, Plate XXIV. Here is shown an arterial branch having a horizontal course, from the under side of which there arise several branches (six) which divide to form arteriolæ rectæ veræ. The preparation is taken from the base of the renal pyramid of the dog's kidney injected with silver nitrate. The structure figured was undoubtedly a small vein receiving several groups of venulæ rectæ, as in corrosion preparations of the venous system of the dog's kidney, such small vessels having a horizontal course and receiving on their under side small branches formed by the union of straight capillaries are frequently met with, but are traceable to larger venous stems. In corrosion

preparations of the rat's kidney, I am able to confirm the observation made by Golubew that the efferent glomerular branches which form the arteriolæ rectæ give off side branches which form capillary networks at the level of the base of the renal pyramid. Such side branches of the efferent glomerular vessels destined to form arteriolæ rectæ I have observed also in the kidneys of the other animals studied, though they are not nearly so numerous as in the white rat. So far as may be determined in corrosion preparations in which the peripheral portions of the interlobular arteries appeared completely injected, these end in afferent glomerular branches and do not present terminal branches which end directly in capillaries in the peripheral portion of the renal cortex. Now and then, and more particularly in the dog, have I found an interlobular branch which did not completely break up into branches within the renal cortex, but could be traced beyond the outer border of the cortex anastomosing, as would appear, with capsular branches. Afferent glomerular branches arise from such interlobular branches to near the peripheral part of the cortex. The question may be asked whether the "arteria capsularis glomerulifera" described by Golubew may not be interlobular arteries of the above type imperfectly injected from the outside. As I have not attempted corrosion injection through the aorta after tying the renal arteries, I am not able to decide this point.

In this account, no mention has been made of observations on the human kidney with reference to the points more particularly under discussion. The limited human material at my disposal has not been fresh enough to enable capillary injection with subsequent corrosion by the method used. In the attempts made the injection mass could readily be forced into the glomeruli, but only to a limited extent into the efferent glomerular vessels, as a rupture of the glomerular vessels in many places allowed its escape into the space enclosed by Bowman's capsule and then into the uriniferous tubules in which it would pass to about the beginning of the descending limb of the loop of Henle, giving excellent and instructive corrosions of the proximal convoluted portions of the uriniferous tubules. Similar observations were made on injecting through the renal vessels of the kidneys of animals several hours after death. It is hoped that this method may prove useful in the hands of others with access to fresh human material in determining the origin of the arteriolæ rectæ and the existence or non-existence of terminal arterial branches not directly associated with glomeruli.

From observations made on corrosion preparations of the dog, cat, rabbit, guinea pig, and rat, in which it is possible to trace the renal

arteries through their several branchings to their termination, including the branches which go to the glomeruli, the glomeruli themselves, the branches leaving the glomeruli, and often the capillary plexuses formed by these, the conclusion seems warranted that practically all of the blood found in the capillaries surrounding the different portions of the uriniferous tubules is blood that has first passed through the glomerular vessels. This was so clearly stated by Bowman[7] many years ago that it seems but just to use his own words to give further emphasis to this point. In Bowman's classical contribution to the anatomy of the kidney is found

Fig. 4. Corrosion preparation of terminal arterial branches of kidney of rabbit.

the statement: "According to my own observations, the circulation through the kidney may be stated to be as follows:—All the blood of the renal artery (with the exception of a small quantity distributed to the capsule, surrounding fat, and the coats of the larger vessels) enters the capillary tufts of the Malpighian bodies; thence it passes into the capillary plexus surrounding the uriniferous tubes and it finally leaves the organ through the branches of the renal vein." With this clear and correct statement of facts, dating back to 1842, it is somewhat surprising that even at the present time, there should be a question as to the existence or non-existence of terminal branches of the renal artery which end in

[7] Bowman: On the Structure and Use of the Malpighian Bodies of the Kidney, with Observations on the Circulation through that Gland. Philosoph. Trans. of the Royal Society of London, 1842, p. 57.

capillaries about uriniferous tubules without being directly associated with glomeruli.

There are, as is well known, two leading and opposing theories on the nature of urinary secretion tersely stated by Hans Meyer as follows in a recent summary of observations on renal function: "According to one of these theories, which was developed most fully by Heidenhain, we have to deal with a true secretory process by which water and perhaps the salts pass through the glomerulus, whereas the specific constituents of the urine are liberated from the tubules, so that the sum of both secretions is represented by the outflowing urine. According to the other hypothesis, which was first proposed by Ludwig and subsequently modified by his successors (in a biological sense), there goes on in the kidney, side by side with the glomerular activity, dependent essentially on the mechanical conditions of the circulation, and independently also on the secretion of certain urinary constituents, a process of resorption in the urinary tubules. Through this resorption the slightly concentrated secretion of the glomerulus, corresponding to the water of the blood, undergoes concentration to a point characteristic of the urine."

It is not my purpose here to discuss either of these theories. It may, however, be permitted to call brief attention to certain points in the structure of the uriniferous tubules in connection with an account of the relations of terminal branches of the renal arteries, points which, it seems to me, should be considered by the followers of either of the leading theories on the nature of urinary secretion.

In each uriniferous tubule, including the glomerular capsule, there may be recognized four types of epithelium with distinct regional distribution. (1) The flattened epithelial cells lining the glomerular capsule continuous with the flattened epithelium, probably syncitial in character covering the glomerulus; (2) the epithelium of the proximal convoluted portion, columnar in shape with striated protoplasm and striated inner border; (3) the peculiar flattened epithelium of the descending limb of Henle's loop; (4) the short columnar epithelium of the ascending limb, the distal convoluted portion and a portion of the junctional tubule, an epithelium with indistinct cell outline, with basal striation, but differing in structural detail and in reaction to stains from the epithelium of the proximal convoluted portion. These four types of epithelium are found, not only in the uriniferous tubules of the mammalian kidney, as determined by reconstruction in this laboratory, but also in the tubules of the simpler reptilian kidney (recently reconstructed in this laboratory and to be described in another communication) as also in the tubules of the amphibian kidney (mesonephros). The epithelium of the neck of the uriniferous tubules is here not especially considered; it differs from the other epithelia described, though it probably has little functional significance. If it is true, as stated by Starling in introducing the section on "The Mechanism of the Secretion of Urine" in Schaefer's Text-book of Physiology, that "a difference of function is invariably associated with a difference of structure, so that the interdependence of function and structure has become an axiom," we should be justified in postulating a difference of function to the different parts of the uriniferous tubules lined by the different types of epithelium, and the extent to which this may be done is, as it

appears to me, briefly as follows: The weight of evidence appears to substantiate the statement that the water, the sodium chloride and urea and probably other substances existing in a free state in the blood are secreted (pass out by filtration or transudation) by the glomerular epithelium. It is estimated that 1-12 to 1-14 of the volume of blood entering through the afferent glomerular vessels is abstracted during the course of the blood through the glomerular vessels, so that the blood leaving the glomeruli is thus proportionately concentrated. To this fact attention may be especially drawn, since, as has been shown, practically all the blood found in the capillaries surrounding the different parts of the uriniferous tubules is blood which has passed through the glomeruli. Uric acid and phosphoric acid appear to be specifically secreted, as their quantity cannot be increased by any of the known diuretics—Hans Meyer. The evidence is in favor of connecting this specific secretion with the epithelium of the proximal convoluted tubules. In confirmation of this may be cited the sodium sulphindigotate experiments of Heidenhain and Ribbert, the carmine injection experiments of Schmidt and Ribbert, the detection of uric acid granules in this epithelium and the presence of what has been regarded as secretory granules in the same epithelium. To what extent the presence of concentrated blood found in the capillaries surrounding the proximal convoluted tubules, having, as may be assumed, a larger per cent of uric acid, since this is apparently not secreted by the glomerular epithelial, favors the secretion of this substance by the epithelium of the proximal convoluted tubules, cannot be stated. The possibility of its doing so may, however, be considered. The experimental evidence appears to favor the view that there is a compensatory resorption of water (probably also certain salts in proportion to their diffusibility or permeability of the renal cells—Cushny) during the passage of the renal secretion through the tubules. That this resorption of water takes place in the loops of Henle is probable from the experiments of Ribbert, and Hausman and Hans Meyer who obtained an increased flow of urine of less concentration after removing the medullary portion of one kidney following extirpation of the other. The suggestion is here made that this resorption, more especially of the water, takes place in the descending limb of Henle's loop, largely owing to the peculiar flattened epithelium possessed by it. That the loops of Henle are longer than generally thought is shown by reconstruction, the larger per cent extending through or nearly through th entire medulla. These segments of the uriniferous tubules are in relation with capillaries conveying concentrated blood, favoring a resorption, since, as has been shown, the arteriolæ rectæ are formed almost without exception by a division of certain of the efferent glomerular vessels. The blood passing to the medulla through the arteriolæ rectæ is returned by the venulæ rectæ, which are, if one may judge by corrosion preparations, much more numerous than the arteriolæ. The loops of Henle are, therefore, in relation with numerous capillaries. Whether special function may be ascribed to the ascending limb of Henle's loop and the distal convoluted portion, which again has a special epithelium, is difficult to state. Heidenhain believed these tubular segments to possess a secretory function similar to that possessed by the proximal convoluted portions, bas-

ing his conclusions on observations made after injecting sodium sulphindigotate. It would seem, however, that absorption of the dye by the epithelium of the ascending limb of Henle's loop and distal convoluted portions, after a concentration as a result of absorption of water in the descending limb is not excluded. Ribbert states distinctly that "a secretion of specific substances takes place only in the convoluted tubules of the first order, while in the loop of Henle, the distal convoluted portion and the collecting tubules, there takes place exclusively or for the greater part a resorption of water." He further draws attention to the fact that in normal kidneys of older individuals there are often found pale yellow granules, contained exclusively in the epithelium of the distal convoluted portions and parts of the loops, and, further, that toxic agents secreted by the kidney affect first the glomeruli and then the distal convoluted portions and in part the loops. It would appear, therefore, that a resorption takes place from these tubular segments perhaps of more specific substances than from the descending limb of Henle's loop.

(Excellent reviews of the literature bearing on renal secretion may be found in a number of recent publications—Ribbert, Untersuchungen über die Normale und Pathologische Physiologie und Anatomie der Niere, Bibliotheka Medica, 1896; Hans Meyer, Herter Lectures, Bull. Johns Hopkins Hospital, Nov. and Dec., 1905; R. Metzner, Die Absonderung und Herausbeförderung des Harnes, Nagel's Handbuch der Physiologie des Menschen, Bd. II, Erste Hälfte, 1906,—to which the interested reader is referred.)

THE PHYLOGENY OF THE PLANTAR MUSCULATURE.

BY

J. PLAYFAIR McMURRICH.

From the Anatomical Laboratory of the University of Michigan.

WITH 9 TEXT FIGURES.

In three papers which have appeared in this JOURNAL I have given the results of a comparative study of the flexor muscles of the antibrachium, hand and crus, and have shown that in each of these parts there is an arrangement of the musculature in definite layers, which can be identified in the amphibia, reptilia and mammalia. And, further, it was shown that there is a close correspondence in the arrangement of the musculature of the antibrachium and crus in the lower forms. There remain to be determined the existence of an arrangement in primary layers in the plantar musculature and the correspondence of these layers with those occurring in the palm. In the present paper I shall consider especially the question of the primary layers of the plantar musculature and their differentiation.

The material which has served for this study consisted of series of transverse sections of the same forms that were employed in my study of the crural flexors, 04, except that, through the courtesy of Dr. M. J. Greenman, Director of the Wistar Institute of Anatomy, I have been able to add to the mammalian series a representative of the Insectivora, *Scaphanus* sp.?, which, unfortunately, however, proved to be of only subordinate value for my purpose, owing to the extensive transformation of the plantar musculature into tendinous structures. I have also had opportunity for studying the plantar muscles of *Iguana tuberculata,* through the courtesy of my colleague, Dr. J. E. Reighard.

I. THE PLANTAR MUSCLES OF THE URODELE AMPHIBIA.

The plantar muscles of *Amblystoma* are arranged in four primary layers, which correspond, layer for layer, with those occurring in the palm. In a transverse section through the foot a little distal to the bases of the metatarsal bones, the arrangement represented in Fig. 1 is seen. Superficially, immediately beneath the integument, is the strong plantar aponeurosis (*pa*), beneath which lies a continuous layer of muscle tissue,

the *flexor brevis superficialis* (*fbs*). Dorsal to this is a layer, consisting at this level of four distinct portions, which is the *flexor brevis medius* (*fbm*); resting directly upon the metatarsals is the third layer, showing indications of division into a number of subordinate portions, and forming the *flexor brevis profundus* (*fbp*); and, finally, extending between the adjacent surfaces of contiguous metatarsals, are the representatives of the fourth layer, the *intermetatarsales* (*im*).

The plantar aponeurosis and flexor brevis superficialis. The plantar aponeurosis is the direct continuation of the strong aponeurosis which covers the muscles of the crus, and over the metatarsals it divides into five slips, which pass to the various digits; the slips to the hallux and

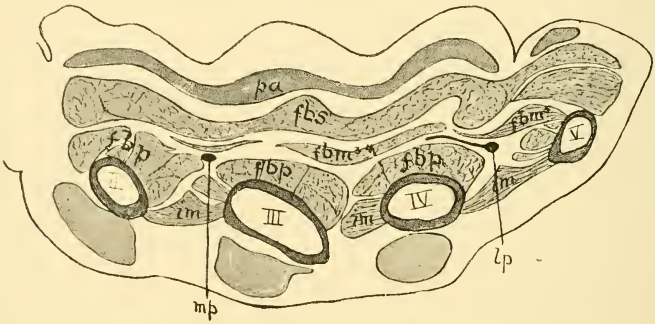


Fig. 1. Transverse section through the foot of Amblystoma. *fbm* = flexor brevis medius; *fbp* = flexor brevis profundus; *fbs* = flexor brevis superficialis; *im* = intermetatarsales; *lp* = lateral plantar nerve; *mp* = medial plantar nerve; *pa* = plantar aponeurosis; *II-V* = metatarsal bones.

minimus had already separated at the level of the section shown in Fig. 1. More proximally, over the tarsals, the aponeurosis receives upon its dorsal surface the insertion of the majority of fibers of the plantares profundi of the crus, these muscles acting on the phalanges through the aponeurosis. In tracing a series of sections from the crus downwards into the foot one finds the plantares gradually diminishing in size as their fibers insert into the aponeurosis, until they are represented only by a few slips which are prolonged further distally than the main masses of the muscles. But just as one begins to expect these slips to completely disappear, they begin to enlarge and more distally form the continuous sheet of muscle which is represented in Fig. 1 as the flexor brevis superficialis, this muscle, accordingly, appearing to be the direct continuation of the plantares pro-

fundi. The continuity is, however, probably merely an apparent one, the fibers of the flexor brevis superficialis beginning to arise from the plantar aponeurosis before those of the plantares profundi have completed their insertion, so that there is a confusion of the two groups of muscles. The fact that one finds, first the continuous sheet of the plantares, then for a short distance three slender slips separated by portions of the plantar aponeurosis, and then again a continuous sheet of flexor brevis superficialis, seems to indicate that one has to do with two distinct muscles, especially when comparison is made with the arrangement in the hand, and when it is noted that the portions of the superficial flexor which pass to the marginal digits arise from the aponeurosis independently of the plantares, the portions continuous with these muscles passing only to the three central digits.

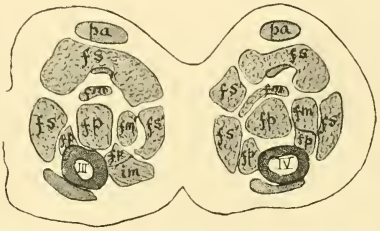


Fig. 2. Transverse section through the metatarsals of the third and fourth digits of Amblystoma near their heads. *fm* and *fm'* = central and lateral slips of flexor brevis medius; *fp* = slips of flexor brevis profundus; *fs* and *fs'* = central and lateral slips of flexor brevis superficialis; *im* = intermetatarsalis; *pa* = plantar aponeurosis; *III* and *IV* = metatarsal bones.

If the plantar aponeurosis and the flexor brevis superficialis be traced distally they will be found to split into as many slips as there are digits, the prolongations of the aponeurosis inserting into the terminal phalanges. In the muscle slips destined for the third and fourth digits the marginal portions (Fig. 2, *fs'*) separate and pass to an insertion into the sides of the heads of their metatarsals, these insertions being closely associated with those of the flexores breves profundi. A little more distally the central portion of each slip (*fs*) begins to undergo a transformation into connective tissue and gives rise to a tendon which applies itself to the dorsal surface of the slip derived from the plantar aponeurosis and fuses with it over the base of the first phalanx, the muscle fibers on either side of this central tendon inserting into the sides of the fibro-cartilages over the metatarso-phalangeal joint.

In the slip to the second digit there is a similar transformation of the median portion into tendon and an insertion of the muscle fibers adjacent to this tendon into the metatarso-phalangeal fibro-cartilages, but there is only one slip passing to the head of the metatarsal, namely, that to the fibular side. The slip to the fifth digit behaves essentially like that to the fourth or third, the only striking difference being the large size of the fibular metatarsal slip; but that to the first digit differs from the rest in that it fails to separate into subordinate slips, but inserts entirely into the metatarso-phalangeal fibro-cartilages.

In addition to the portions of the flexor brevis superficialis described above, another portion is probably represented by the *abductor quinti digiti*, or, as it may be more accurately termed, the *abductor ossis metatarsi V.*, which arises from the fibular border of the tarsus and inserts into the base of the fifth metatarsal, a sesamoid cartilage being developed at its insertion.

The *flexor brevis medius* takes its origin from the aponeurotic layer which lies immediately dorsal to the plantares profundi. It appears as four distinct slips, one of which (Fig. 1, *fbm*$^{3\,4}$) later divides, so that there is a slip for each digit. Toward the distal ends of the metatarsals the slips which pass to the third, fourth, and fifth digits divide into two portions, one of which (Fig. 2, *fm*), much smaller than the other, lies upon the plantar surface of the median slip of the corresponding flexor brevis profundus, while the other portion (*fm'*) rests upon the fibular slip of the same muscle. This latter portion inserts into the side of the head of its metatarsal, in more or less close association with the fibular slip of the flexor brevis profundus, and the smaller portion inserts into the metatarso-phalangeal fibro-cartilage. The slips to the second and first digits do not divide in this manner, but insert entirely into the articular fibro-cartilages.

The *flexor brevis profundus* is composed of three slips for each digit, a median and two lateral (Figs. 1 and 2). The lateral slips arise from the tarsal bones, and, in the cases of the marginal digits, partly from the plantar aponeurosis. The median slip, on the other hand, arises from the plantar surface of its metatarsal, and in the central digits separates the lateral slips, which, up to the level of its appearance, form a single mass. The lateral slips insert into the heads of the metatarsals, the fibular slips of the four tibial digits being intimately associated with the intermetatarsals, and the same slips of the third, fourth and fifth digits with the fibular slips of the flexor brevis medius for those digits. The median slips, which are the metatarso-phalangei of Humphry, 72, extend further dis-

tally and insert in all five digits into the metatarso-phalangeal fibro-cartilages.

In the third and fourth digits inter-phalangeal muscles, the phalangei of Humphry, also occur, passing from the plantar surface of the proximal phalanx to the base of the second one.

The *intermetatarsales* (Fig. 1, *im*), extend obliquely across the inter-metatarsal spaces from the fibular to the tibial side. They are four in number, arising from the tibial sides of the bases of the second, third, fourth and fifth metatarsals, and inserting into the fibular sides of the heads of the first, second, third and fourth metatarsals in association with the fibular slips of the flexor brevis profundus of those digits.

The lateral plantar nerve is, as I have shown elsewhere, **04**, the continuation into the foot of the ramus superficialis fibularis of the crus, while the medial plantar is the continuation of the ramus profundus.

In the proximal tarsal region the lateral plantar nerve lies immediately upon the fibular border of the fibulare and the medial plantar upon the centrale. When the flexores brevi profundi appear they lie between the nerves and the bones, and still more distally, after the flexores breves medii have appeared, the nerves are situated between these muscles and the flexores breves profundi, the medial plantar over the interspace between the second and third metatarsals and the lateral plantar over that between the fourth and fifth (Fig. 1, *mp* and *lp*). The medial nerve gives off branches both medially and laterally, the lateral one meeting a medially directed branch from the lateral plantar opposite the inter-space between the third and fourth metatarsals, so that it becomes difficult to determine from which of the two nerves the branches to the muscles arise. It would seem, however, that the lateral plantar supplies all the muscles of the fifth digit and those inserting into the fibular side of the fourth, while the remaining plantar muscles are supplied by the medial nerve. Certain it is that the terminal cutaneous branches of the two nerves are distributed in such a way that the contiguous surfaces of the four tibial digits are supplied by the medial plantar and those of the fourth and fifth digits by the lateral plantar, the lateral surface of the minimus and the medial surface of the hallux being supplied by branches which descend from the crus.

This distribution differs materially from that described by Humphry, **72**, for Cryptobranchus. In that form the lateral plantar was found contributing to the supply of the third and second digits. In amblystoma it does not extend tibially beyond the fourth digit, the intermetatarsal between the fourth and third digits, for instance, being supplied by the medial plantar.

II. The Plantar Muscles of the Lacertilia.

The manus of the lacertilia compared with that of the urodeles showed a considerable increase in the number of muscle layers, the four urodelan layers being represented by seven. In the pes a similar increase occurs, but it is not carried to quite the same extent as in the manus, the flexor brevis medius layer being divided into only two layers instead of three.

In a previous paper, 04, I showed that the aponeurosis of the crural flexors is, in the lacertilia, divided into a superficial and a deeper layer. The superficial layer is continued into the planta as a well marked aponeurosis (Fig. 3, pa_s) intervening between the integument and the flexor brevis superficialis, and contains several thickened bands which pass to

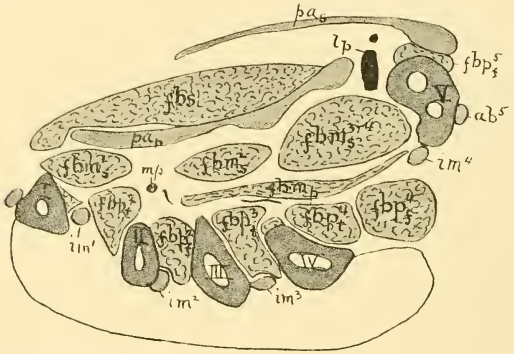


Fig. 3. Transverse section through the foot of Scincus, near the bases of the metatarsals. ab^5 = abductor quinti digiti; fbm_p = flexor brevis medius str. profundum; fbm_s = flexor brevis medius str. superficiale; fbp_t and fbp = fibular and tibial slips of flexor brevis profundus; fbs = flexor brevis superficialis str. superficiale; im = intermetatarsal ligaments; lp = lateral plantar nerve; mp = medial plantar nerve; pa_s and pa_p = superficial and deep layers of the plantar aponeurosis.

the digits and insert with the tendons of the flexor brevis superficialis. The layer is especially developed towards the fibular side of the foot, passing in Scincus to all the digits except the first, but in Iguana being limited to the third, fourth and fifth, only an exceedingly thin layer of fascia covering the muscles passing to the first and second digits. The slip to the minimus is a strong triangular sheet which easily separates from the rest of the aponeurosis.

The *flexor brevis superficialis* (Fig. 3, *fbs*) lies immediately beneath the superficial plantar aponeurosis and consists of a stratum superficiale

and a stratum profunduf. The *stratum superficiale* (Fig. 4, fb_s), takes its origin from the sesamoid bone developed in the tendon of the crural plantaris profundus II-III, and, therefore, from a portion of the superficial aponeurosis. In Scincus it forms a continuous sheet, lying at first to the medial side of the terminal portion of the plantaris superficialis lateralis and resting directly on the continuation of the tendon of the plantaris profundus II-III. As this tendon divides into slips for the five digits, the flexor superficialis divides into corresponding portions, each of these, as a rule, again dividing into two slips, which insert into either side of the base of the proximal phalanx, the tendon of the plantaris profundus II-III passing between them. The slip to the hallux could not be traced

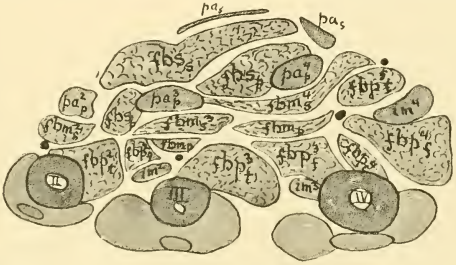


Fig. 4. Transverse section through the foot of Scincus near the heads of the second metatarsal. fbm_p = flexor brevis medius str. profundum; fbm_s = flexor brevis medius str. superficiale; fbp_f and fbp_t = fibular and tibial slips of flexor brevis profundus; fbs_p = flexor brevis superficialis str. profundum; fbs_s = flexor brevis superficialis str. superficiale; im = intermetatarsal ligaments; pa_p and pa_s = deep and superficial layers of the plantar aponeurosis; *II-IV* = metatarsals.

to the phalanx, but faded out over the tendon of the plantaris before reaching the metatarso-phalangeal joint.

In Iguana the muscle, though well developed, is limited in its insertion to the three tibial digits, the slip for the hallux early separating from the rest of the muscle and no slips passing to either the fourth or the fifth digit.

In addition to the flexor brevis superficialis the abductor quinti digiti (Fig. 3, ab^5) is probably to be assigned to the superficial plantar layer. In Scincus it is a small muscle which arises from the surface of a strong ligament extending from the fibular surface of the proximal tarsal bone to the base of the fifth metatarsal. As was the case with the corresponding muscle in the urodeles, its assignment to the superficial plantar layer is not beyond question, although it is indicated by the position of the muscle

and by the fact that it is supplied by a branch given off from the lateral plantar nerve before it bends dorsally to reach its final position between the middle and deep layers of flexors.

The *stratum profundum* (Fig. 4, fbs_p) of the flexor superficialis is represented by two muscles which take their origin from the plantar surface of the tendon of the plantaris profundus II-III before it separates into its terminal slips. The muscle lies in the intervals between the second and third and third and fourth of these terminal slips, and some of their fibers arise from the slips passing to the third and fourth digits. The more tibial muscle is directed fibularly in its distal course, and, passing over into a tendon, is inserted into the tibial side of the base of the proximal phalanx of the third digit, in close proximity to the slip of the flexor brevis medius str. superficiale to that digit. The more fibular muscle has almost the same relations, except that it fuses with the large slip of the flexor brevis medius str. superficiale to the fourth digit, forming a muscular mass which completely invests the plantaris profundus III-II tendon to the digit. The relations of the muscles are practically identical in both Scincus and Iguana; they seem to correspond to the muscles η and ζ of Gadow's, 82, second layer and to those numbered 17 and 18 by Perrin, 93.

The *flexor brevis medius* is represented by two distinct muscle layers. The *stratum superficiale* (Figs. 3 and 4, fbm_s), lies immediately dorsal to the tendons of the plantaris profundus III-II, and in Scincus consists at its origin of three portions. The tibial and middle portions arise from the plantar surface of the base of the second and fourth metatarsals and from the connective tissue covering those bones, while the fibular portion, much the strongest of the three, has an extensive origin from the fibular border of the fifth metatarsal. The tibial and fibular portions retain their individuality throughout, the former passing distally and tibially to be inserted into the first metatarso-phalangeal fibro-cartilage, while the latter passes to the corresponding structure of the fourth digit. The middle portion divides into two slips, which pass respectively to the metatarso-phalangeal fibro-cartilages of the second and third digits (Fig. 4, $fbm_s{}^{3\,4}$).

In Iguana the arrangement of the layer is essentially the same as in Scincus, although there are some differences in detail. The muscles take origin in part from the tarsal bones, instead of from the metatarsals, and in part receive numerous fibers from the dorsal surfaces of the tendons of the plantaris profundus III-II. The tibial portion is strong and quite independent of the others; the median portion, which

has a considerable origin from the dorsal surface of the plantaris tendon as well as from the tarsus, passes to the second and third digits; while the fibular portion, which is strong, as it approaches the fourth metatarso-phalangeal joint, invests the fourth plantaris tendon and comes into such intimate connection with the fibular slip of the flexor brevis superficialis str. profundum as to be unseparable from it.

This layer seems to correspond to Gadow's, 82, second plantar layer (less the slips η and ζ already referred to the flexor brevis superficialis str. profundum) together with slip α of his third layer. His second layer contains no slip to the hallux, while his third layer possesses two slips to that digit, one of which presents an appearance and arrangement similar to the hallucal slip of the flexor brevis medius str. superficiale of Scincus and Iguana. The same slip is described by Hoffmann, 90, as the tarso-digitalis primus and by Perrin, 93, as No. 30 flexor of the first phalanx. The latter author describes the middle slip as 9i, tarso-flexor of the digits, and the fibular slip as the external portion of 18, flexor of the fourth phalanx, the internal portion of that muscle being the slip of the flexor brevis superficialis str. profundum to the fourth digit.

The *flexor brevis medius stratum profundum* (Figs. 3 and 4, fbm_p) is a thin sheet which lies immediately dorsal to the str. superficiale and is separated from the flexores breves profundi by the deep branches of the plantar nerves. It arises from the bases of the fifth and fourth metatarsals and, to a certain extent, from that of the third, and its fibers are directed obliquely distally and tibially. It divides over the shafts of the metatarsals into four slips which pass to the fibular side of the metatarso-phalangeal fibro-cartilage of the first, second, third, and fourth digits. This muscle corresponds to the third plantar layer of Gadow, 82, with the omission of slip α, and to the deductors of Perrin, 93.

The flexores breves profundi (Figs. 3 and 4, *fbp*). These muscles form a layer resting directly upon the metatarsals and separated from the flexor brevis str. profundum by the deep branches of the plantar nerves. They form in Scincus ten slips, which do not, however, correspond by pairs to the five digits. So far as the three tibial digits are concerned a paired arrangement is clear, although the muscles for each digit arise from the next adjacent metatarsal. The fourth digit, however, has three slips attached to it, and the fifth only one, an arrangement which may indicate a transference of one of a pair corresponding to the fifth digit to the fourth.

Of the slips which pass to the metatarso-phalangeal fibro-cartilages of the first, second, and third digits, one (fbp_t) arises from the tibial surface and the other (fbp_f) from the plantar surface of the next adjacent metacarpal on the fibular side. In the case of the fourth digit one slip (Fig. 4, $fbp^4{}_t$) arises from a strong ligament which extends distally from the cuboid; a second slip ($fbp^4{}_f$) arises partly from this same ligament, which, much reduced in size, accompanies it throughout its course, and partly from the tibial surface of the fifth metatarsal; while the third ($fbp^5{}_t$) takes its origin from the head of the fifth metatarsal in close association with the intermetatarsal ligament. All three slips insert into the metatarso-phalangeal fibro-cartilages of the fourth digit.

The third slip from its position might readily be interpreted as a portion of the flexor brevis medius, stratum profundum. The fourth digit, however, has another slip which is plainly a part of that layer, and, furthermore, the deep branch of the lateral plantar nerve passes to its deep position between the slip under discussion and the flexor brevis medius, the slip, therefore, lying practically dorsal to the nerve layer and having the same relation to it as the other deep flexors. Its identification as one of these makes it seem probable that it really represents one of a fifth pair, its fellow being a slip (Fig. 3, $fbp^5{}_f$), which arises from a ligament extending between the talo-calcaneus and the base of the fifth metatarsal. It passes distally upon the fibular surface of the metatarsal, parallel to the lower part of the plantaris superficialis lateralis, from which it is separated by the lateral plantar nerve, and inserts into the fifth metatarsal near its distal extremity.

In Iguana I find essentially the same arrangement of the flexores breves profundi, although there are slight differences in detail. The first and second digits each receive two slips, but the slip to the third digit could not be divided into two portions. It arose, however, partly from the tibial surface and partly from the plantar surface of the fourth metatarsal and consequently agreed with the two slips found in Scincus. To the fourth digit three slips can be distinguished, of which that corresponding to $fbp^4{}_f$ is much the most prominent and completely conceals $fbp^4{}_t$, which is represented by a narrow and thin band of fibers, inseparable at its origin from $fbp^3{}_f$, although diverging from it distally. The third slip, which arises from the base of the fifth metatarsal, is quite small and after a short course unites with the fourth intermetatarsal. The single slip to the fifth digit is much stronger than in Scincus, covering the whole plantar surface of the metatarsal and having upon it in the median line the tendon of the flexor plantaris profundus.

The *intermetatarsales.* In Scincus and Iguana these muscles have the same structure and relations as the corresponding muscles of the hand. They are represented by four slender tendons (Figs. 3 and 4 *im*) which pass to the bases of the proximal phalanges of certain digits from certain metatarsals. The first tendon passes from the first metatarsal to the phalanx of the second digit; the second from the second metatarsal to the phalanx of the third digit; the third from the third metatarsal to the phalanx of the fourth digit; and the fourth from the fifth metatarsal to the phalanx of the fourth digit. Consequently the fourth digit receives the insertion of two of the tendons and the second and third digits each receive one.

The nerve supply of the plantar region presents some interesting differences from what obtains in the urodeles. At the level of the ankle joint the medial plantar nerve or ramus profundus is situated deeply, resting upon the talo-calcaneus dorsal to the plantaris profundus I, while the lateral plantar or ramus superficialis fibularis lies upon the dorsal surface of the plantaris superficialis lateralis and has, therefore, a plantar position with reference to the plantares profundi (see Fig. 5 of my paper on the crural flexors, 04). The medial plantar retains its deep position as it is traced onwards into the foot, the flexores breves profundi, however, appearing between the nerve and the metatarsals so that the nerve comes to lie between these muscles and the flexor brevis medius str. profundum over the line of the second metatarsal (Fig. 3, *mp*). Over the proximal half of that bone it gives off a branch which is supplied to the various slips of the flexor medius and profundus sets of muscles which are inserted into the hallux, apparently also to the slip of the flexor superficialis which passes to that digit, and is finally distributed to the adjacent sides of the first and second digits. The remainder of the nerve continues its distal course, bending slightly towards the fibular border of the foot so that it comes to lie at first over the second intermetatarsal space and then over the fibular border of the third metatarsal. It gives off a branch to the fibular side of the second digit and toward the head of the third metatarsal it divides into two terminal branches which supply the sides of the third digit. The peculiar condition is thus produced that the muscular distribution of the nerve is confined to the muscles inserting into the hallux, while its cutaneous distribution extends over the three tibial digits.

The lateral plantar behaves quite differently. Beyond the ankle joint it continues its course lying over the fifth metatarsal between the terminal portion of the plantaris superficialis lateralis and the abductor quinti

digiti; but just opposite the insertion of the latter muscle it bends dorsally (Fig. 3, *lp*), passing between the terminal portion of the abductor on the one side and the hallucal long flexor tendon and slip of the flexor brevis superficialis in the other, giving off at the same time two branches. One of these remains superficial and is continued along the medial border of the fifth digit, while the other passes dorsally with the main stem of the nerve and then bends medially to be supplied to all the slips of the flexor brevis medius str. superficiale except that which passes to the hallux. The main stem when it reaches the interval between the flexor brevis medius str. profundum and the flexores breves profundi (Fig. 4) makes an abrupt bend and passes medially as far as the line of the second metatarsal, passing dorsal to the main stem of the medial plantar. Without going into details regarding the various branches given off by the nerve in this deep portion of its course, it may be said that it supplies all the portions of the flexor brevis medius and flexor profundus layers except those which pass to the first digit, and that its cutaneous distribution is limited to the fourth and fifth digits.

This condition is very different from what occurs in Amblystoma, in which the medial plantar has the major supply of the pes. It would seem that there has been a shifting of fibers from the profundus to the fibular superficial stem, so that muscles originally supplied by the former are, in the lacertilia, supplied by the latter, and it is interesting to note that the transference has taken place to a much greater extent in connection with the motor fibers than with the cutaneous ones, so that the sensory supply of the medial plantar extends to digits where muscles are entirely supplied by the lateral plantar.

In making a comparison between the muscles of the urodele and lacertilian it is evident that the nerve supply fails to give any criterion for homology and there is left only the evidence from topographic relations. This, however, yields results which seem conclusive.

The homologies may be briefly stated in the form of a table, no discussion seeming to be necessary except in regard to the flexor brevis medius. In this muscle, as has been noted, there are two layers in the lacertilia, while only one was recognized in the amphibia. There seems little room for doubt, however, that indications of the double layering exist in the urodeles, each slip of the medius in these forms dividing into a more tibial and smaller portion and a larger fibular portion. These lie practically side by side and therefore do not represent exactly the condition in the lacertilia, but nevertheless it seems probable that the tibial

slips are the urodele equivalents of the lacertilian stratum superficiale. If this view be adopted the homologies of the muscles in the two groups may be tabulated as follows:

Urodeles.	*Lacertilia.*
Flexor brevis superficialis	Flexor brevis superficialis stratum superficiale. Flexor brevis superficialis stratum profundum.
Flexor brevis medius fasciculus tibialis.	Flexor brevis medius stratum superficiale.
Flexor brevis medius fasciculus fibularis.	Flexor brevis medius stratum profundum.
Flexores breves profundi.	Flexores breves profundi.
Intermetatarsales.	Ligg. intermetatarsalia.

III. The Plantar Muscles of the Mammalia.

In 1878 Ruge published two important papers dealing with the plantar muscles of the mammalia, one, **78**, being a consideration of the muscles of the human foot from the embryological standpoint, and the second, **78a**, a comparative study of the deeper plantar muscles. In the first paper two important results were recorded, namely, (1) the plantar nature of both the dorsal and plantar interossei, and (2) the primary unity of the adductor hallucis and the transversus pedis. In the second paper, disregarding the superficial muscles and relying upon the doctrine of the immutability of the nerve supply of muscles, the author separates these muscles which are supplied by the medial plantar nerve from those innervated by the lateral plantar, and divides the latter into two groups, one of which the contrahentes, lies to the plantar side of the deep branch of the lateral plantar nerve, while the other, formed by the interossei, lies dorsal to the nerve.

In the same year that Ruge's papers appeared Cunningham, **78a**, published the results of his extensive comparative studies of the plantar muscles, furnishing later, **82**, a more detailed account of his observations. Like Ruge, he disregarded the superficial muscles, but, on the other hand, he declined to accept nerve supply as an absolute criterion for muscle homology, and found in what he termed the "intrinsic" muscles, representatives of the same three layers he had already demonstrated, **78**, in the hand. The most superficial to these layers lies dorsal to the tendons of the long flexor and plantar to the deep branch of the lateral plantar nerve; it is termed the plantar layer of adductors

and corresponds to Ruge's contrahentes. The second and third layers lie dorsal to the nerve and together correspond to Ruge's layer of interossei; the more superficial muscles Cunningham termed the intermediate layer of flexores breves and the deeper one the dorsal layer of abductors.

In the urodeles and lacertilia I have shown that three (or four) layers are distinctly recognizable dorsal to the long flexor tendons or their homologue, and for this reason Cunningham's arrangement of the deep muscles is preferable to that of Ruge. His dorsal layer of abductors is in part equivalent to what have been described in the preceding pages of this paper as the intermetatarsales; his intermediate layer is similarly equivalent in general to my flexores breves profundi; while his plantar layer of adductors corresponds to my flexor brevis medius str. profundum. But by the exclusion of the superficial layers—indeed of everything superficial to and connected with the long flexor tendons, both Cunningham and Ruge fall into error in the assignment to their "intrinsic" or deep muscles of certain structures which are derivatives of the superficial layer. There are in the foot two superficial layers of muscles which are just as properly termed intrinsic as are the adductors and interossei, and in what follows I shall recognize the same layers as have been described in the foot of the lower forms, giving special names to certain of the marginal muscles when this seems desirable.

The *flexor brevis superficialis.* In the mammalia studied the flexor brevis superficialis was represented by both the strata described in the lacertilia. In the *superficial stratum* of the lacertilia there was a distinct tendency for the muscle slip for the hallux to separate from the rest of the layer; this becomes more pronounced in the mammals, in which the slip becomes practically an independent muscle. Furthermore, we must include in this layer certain marginal muscles of the foot, which may be termed abductors.

Considering first the main portion of the muscle, it arises in the opossum from the plantar surface of the strong tendon of the flexor fibularis cruris, a short distance above the ankle joint, and partly also from the tendon of the flexor tibialis at about opposite the ankle joint, and is distributed by four slips (Figs. 5 and 6, fbs_s), to the second, third, fourth, and fifth digits, each slip dividing into two tendons, between which passes a tendon of the long flexor. In the cat and mouse the fibers arise from the aponeurosis into which the long flexors insert. The difference, however, is more apparent than real, since, as has already been pointed out, McMurrich, 04, both the aponeurosis and the tendons of the long flexors represent portions of an original plantar aponeurosis.

The well developed slip of the flexor brevis superficialis which passed to the hallux in the lacertilia is represented in the opossum by two closely associated muscles. One of these, which may be termed the *abductor hallucis* (Fig. 5, *ah*), arises from the dorsal surface of the tarsal spur, over the base of the first metatarsal, and is inserted into the first phalanx in close proximity to the medial metatarso-phalangeal sesamoid cartilage; the other, which may be designated the *flexor brevis superficialis hallucis* (Fig. 5, *fbs'*), arises in part from the dorsal surface of the tarsal spur, but more extensively from the sheath which encloses the tendon of the long flexor, the main portion of the flexor brevis superficialis and the medial plantar nerve, and is inserted into the medial metatarso-phalangeal sesamoid cartilage.

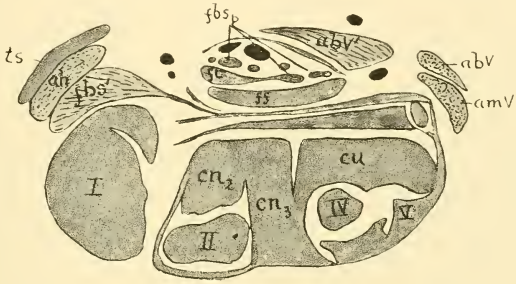


Fig. 5. Transverse section through the junction of tarsus and metatarsus in the opossum. *abV* and *abV'* = abductor quinti digiti; *ah* = abductor hallucis; *amV* = abductor ossis metatarsi quinti digiti; cn_2 and cn_3 = second and third cuneiforms; *cu* = cuboid; *fbs'* = hallucal slip of flexor brevis superficialis; fbs_p = flexor brevis superficialis str. profundum; *ff* = tendon of flexor fibularis; *ft* = tendon of flexor tibialis; *ts* = tarsal spur; *I*, *II*, *IV*, and *V* = metatarsals.

These two muscles, although closely associated, are separated by a distinct sheet of connective tissue, and their fibers have a markedly different direction, so that in sections they are readily distinguishable. Considerable differences of opinion have been expressed as to their significance. Coues, **72**, failed to recognize the abductor as distinct from the more lateral slip, which he describes as a portion of the flexor brevis hallucis; Ruge, **78a**, who fails to recognize two muscles, terming the combination the abductor hallucis; while Cunningham, **82**, who does recognize the abductor, refers it to his dorsal layer (*i. e.*, the intermetatarsal layer), while the flexor superficialis hallucis he assigns as part of the flexor

brevis hallucis to his intermediate layer (*i. e.*, the flexores breves profundi layer). The fact that both muscles are supplied by the medial plantar nerve and their apparent equivalency to the strong hallucal slip of the lacertilian flexor brevis superficialis lead me to regard Cunningham's assignments as incorrect; they are due to the erroneous conception of the intrinsic muscles adopted by Cunningham.

In the mouse, and to a greater extent in the cat, there is a reduction of the hallucal muscles owing to the reduction of the digit. In the mouse a muscle, supplied by the medial plantar nerve, arises from the navicular bone and is directed medially and distally to be inserted into the tibial side of the first phalanx of the hallux. It represents the hallucal slip of the flexor brevis superficialis, and in two out of three individuals studied was a single muscle. In a third individual, however, it had two heads, one from the navicular and the other, much smaller, from the tibial sesamoid bone which Baur, **85**, has identified with the tibiale. The two heads eventually fuse, but it seems not improbable that the smaller one represents the abductor hallucis.

In the cat the only representative of a superficial hallucal flexor seems to be the muscle named scapho-cuneiformis by Reighard and Jennings, **01**. It arises from the navicular bone and from the calcaneo-cuneiform ligament and, in the individual studied, could be distinctly traced to the base of the rudimentary metatarsal. Reighard and Jennings describe it as inserting into the lateral surface of the first cuneiform; the difference may be due to my preparations having been made from an advanced fetus. The muscle is supplied by a branch from the medial plantar nerve.

The fifth digit, in addition to a slip from the main mass of the flexor brevis superficialis, receives certain other superficial muscles which must be assigned to that layer. In the opossum there are three such muscles. One, for which Cunningham's, **82**, name *abductor ossis metatarsi quinti digiti* (Fig. 5, *amV*), may be adopted, has its origin from the lateral surface of the tuberosity of the calcaneus and passes distally, over the quadratus plantæ to be inserted into the lateral surface of the base of the fifth metatarsal. A second muscle (Fig. 5, *abV*), arises from the calcaneus in close proximity to the preceding and over the base of the fifth metatarsal is continued into a slender tendon, which inserts into the lateral surface of the base of the proximal phalanx of the digit; while the third muscle (Figs. 5 and 6, *abV'*), takes its origin from the plantar surface of the sheath enclosing the long flexor tendons and passes distally and laterally to unite with the tendon of the second muscle near its

insertion. These last two muscles, Cunningham, **82**, describes as a two-headed abductor quinti digiti. All three muscles are supplied by branches from the lateral plantar nerve.

Ruge, **78a**, describes the same three muscles, but Coues, **72**, failed to find the abductor ossis metatarsi, the arrangement described by him being similar to that observed by Ruge in *Didelphys cancrivorus*. Furthermore, Coues terms the oblique head of the abductor the flexor brevis minimi digiti, an objectionable term for it on account of its leading to a confusion both with the slip which passes to the minimus from the main mass of the flexor brevis superficialis and with the homologue of the muscle known by the same name in human anatomy (see p. 430). While Cunningham's terminology for the muscles is acceptable, it may be noted that here, as in the case of the abductor hallucis, he is in error in referring them to his dorsal layer.

In the lists which Cunningham, **82**, gives of the muscles of his dorsal layer as they occur in the large number of mammals he studied, it will be noticed that while the three muscles occur in several marsupials, in other forms they are reduced to two and in others to one. When two exist they are a single-headed abductor and an abductor ossis metatarsi; and when but one occurs it may be either an abductor or an abductor ossis metatarsi. The cat belongs to that group of forms in which there are two muscles, an abductor ossis metatarsi (the calcaneo-metatarsalis of Reighard and Jennings, 01), passing from the side of the tuberosity of the calcaneus to the base of the fifth metatarsal, and an abductor (the abductor medius quinti digiti of Reighard and Jennings), arising from the plantar aponeurosis over the abductor ossis metatarsi and inserting into the lateral metatarso-phalangeal sesamoid bone. The mouse, on the other hand, possesses only an abductor ossis metatarsi. The muscles of both forms are supplied by branches from the lateral plantar nerve.

The *flexor brevis superficialis stratum profundum* is represented in all the mammalia studied by muscle fibers which are distinctly separated from those of the stratum superficiale and take their origin from the plantar surface of the long flexor tendon shortly before it divides into its slips for the digits. In the opossum the muscle forms a practically continuous sheet, covering the entire width of the tendon, and divides distally into four slips (Figs. 5 and 6, fbs_p), one of which passes to each of the tendons of the main mass of the stratum superficiale. In the cat (Fig. 7, fbs_p) and mouse the stratum is represented by only two slips which unite with the superficial tendons for the third and fourth digits. Those occurring in the cat have been described by Reighard and Jen-

nings, 01, as lumbricales, but they cannot properly be regarded as belonging to that group of muscles.

The *flexor brevis medius stratum superficiale.* This layer is represented in the mammals in part by the lumbricales. In the opossum these are four in number (Fig. 6, fbm_s), arising from the tendon of the flexor fibularis just as it divides into the tendons for the four lateral digits. Three of the muscles consequently arise in the angle between the four diverging tendons and the fourth from the tibial side of the tendon to the index. The muscles pass to the tibial side of the base of the proximal phalanx of the second, third, fourth, and fifth digits. In the mouse there are also four lumbricals arising in the angles formed by the split-

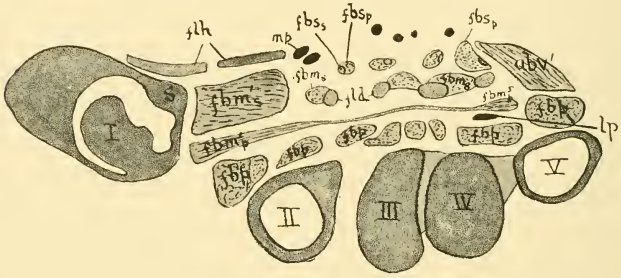


Fig. 6. Transverse section through the foot of the opossum. abV' = abductor quinti digiti; fbm_p = flexor brevis medius str. profundum; fbm_s = flexor brevis medius str. superficiale; fbp = flexor brevis profundus; fbs_p = flexor brevis superficialis str. profundum; fbs_s = flexor brevis superficialis str. superficiale; fld = tendon of flexor longus digitorum; flh = tendon of flexor longus hallucis; lp = lateral plantar nerve; mp = medial plantar nerve; s = metatarso-phalangeal sesamoid cartilage of hallux; I-V = metatarsals.

ting of the long flexor tendon and passing to the same digits as in the opossum, while in the cat there are only three muscles in the group, that for the second digit being lacking.

In the opossum and mouse the muscle to the second digit is supplied by a branch of the medial plantar nerve, while the other three are supplied by the lateral plantar. In the cat all the muscles of the set are supplied by the lateral plantar, the muscle to the second digit being, as stated, wanting.

In the lacertilia a large muscle belonging to the superficial layer of the flexor brevis medius passes to the hallux, and in the opossum what I believe to be the same muscle (Fig. 6, fbm'_s) occurs. It arises in close association with the hallucal portion of the flexor brevis superficialis,

from the medial surface of the sheath enclosing the long flexor tendons, and also from the base of the second metatarsal. It passes distally parallel with the hallucal slip of the flexor brevis superficialis, the long flexor tendon for the hallux lying between the two muscles, and is inserted into the lateral surface of the base of the first phalanx, a sesamoid cartilage being developed in its tendon. It is supplied by the deep branch of the lateral plantar nerve. In the cat and the mouse the muscle does not occur, unless it be represented in the mouse by a few scattered muscle fibers which occur in new born animals between the hallucal slips of the flexor brevis superficialis and the flexor brevis medius str. profundum.

This is the muscle which Coues, 72, and Cunningham, 82, describe as the lateral head of the flexor brevis hallucis associating it with the hallucal slip of the flexor brevis superficialis, the latter author referring both slips to his intermediate layer (*i. e.*, the flexor brevis profundus). Ruge, 78a, on the other hand, regards the muscle as distinct from the flexor brevis superficialis slip and refers it to his layer of contrahentes (*i. e.*, to the flexor brevis medius str. profundum). I shall have occasion to consider this muscle or rather its human equivalent in connection with the human flexor brevis hallucis and shall remark concerning it here only that it occupies a plane ventral (*i. e.*, plantar) to that of the hallucal portion of the flexor brevis medius str. profundum (Fig. 6) and that this fact, together with its innervation from the lateral plantar nerve and the relations of what is apparently the corresponding muscle in the lacertilia, lead me to consider it a portion of the superficial layer of the flexor brevis medius.

The *flexor brevis medius stratum profundum* is formed by what are usually known as the adductors or, as they have been termed by Ruge, 78a, following Bischoff, 70, the contrahentes. In the opossum they are four in number. The two muscles which pass respectively to the hallux and minimus (Fig. 6, $fbm_p^{1\,5}$) are large fan-shaped structures which arise from a median tendinous raphe extending from the base of the third metatarsal to the base of the proximal phalanx of the third digit, the fibers converging from this raphe on the one side to the base of the proximal phalanx of the hallux and on the other to the base of the proximal phalanx of the minimus. The other two muscles are almost concealed by those just described, beneath which they lie, taking their origin from the dorsal surface of the tendinous raphe and passing distally to be inserted, the one into the fibular side of the base of the second digit and the other into the tibial side of the base of the fourth digit. All four muscles are supplied by branches from the deep branch of the lateral plantar nerve.

In the mouse the layer is represented by only three muscles, that to the fourth digit being wanting. They arise from the strong fibrous sheath which invests the peroneus longus and insert into the fibular side of the bases of the proximal phalanges of the first and second digits and into the tibial side of the corresponding phalanx of the minimus. In the cat the reduction in number is carried one step farther, in that the muscles pass to only two digits, namely the index and minimus (Fig. 7, $fbm_p^{2\,5}$), but the latter digit receives two slips, one of which is inserted into the tibial side of the base of the proximal phalanx, while the other inserts into the tibial side of the metatarsal near its head and forms what has been termed the *opponens minimi digiti*. In both forms all the muscles are supplied from the lateral plantar nerve.

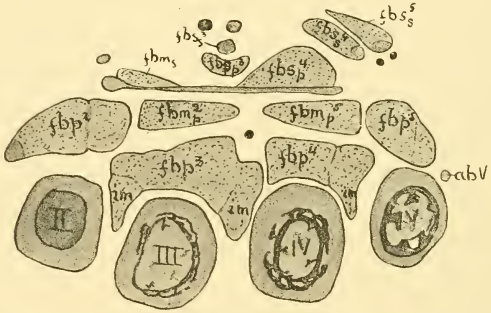


Fig. 7. Transverse section through the foot of the cat. *abV* = abductor quinti digiti; fbm_p = flexor brevis medius str. profundum; fbm_s = flexor brevis medius str. superficiale; *fbp* = flexor brevis profundus; fbs_p = flexor brevis superficialis str. profundum; *fbs* = flexor brevis superficialis$_s$ str. superficiale; *im* = intermetatarsalis; *II-V* = metatarsal bones.

The *flexores breves profundi* and the *intermetatarsales* are so intimately associated in all the forms studied that they may be considered together. Compared with the lacertilia it is noticeable that the intermetatarsales are represented by muscles, instead of ligaments, and that there is a very different arrangement of the flexores profundi with reference to the various digits. Instead of a general inclination of the slips from the fibular to the tibial side one finds that in the mammalia they are almost directly longitudinal in their course and that certain of them are so fused with the intermetatarsales as to be distinguishable from them only by intervening fibrous bands, in some cases by a more or less pronounced difference in the direction of their fibers and by their more plantar position.

In the opossum there occurs in the interval between the first and second metatarsals a muscle (Fig. 6) which arises from the base of the first metatarsal and passes distally to be inserted into the tibial metatarso-phalangeal sesamoid cartilage of the second digit. In cross sections near its origin the muscle is distinctly seen to be composed of two portions, separated from one another by a tendinous partition, and the fibers on either side of the partition have a distinctly different direction. How far the presence of the partition and the difference in direction may be relied upon as an indication of a fusion of two primarily distinct muscles is uncertain, especially since towards their insertion the two portions fuse so as to be indistinguishable. I am inclined to believe, however, on the basis of comparison with lower forms, that in this case the peculiarities do indicate a fusion and that the muscle really represents a flexor brevis profundus hallucis and an intermetatarsalis I.

Passing fibularly one finds over the second metatarsal two muscles again separated by a tendinous partition and also showing a very different direction of their fibers. These are evidently the flexores breves profundi II and they insert into the two sesamoid bones over the metatarso-phalangeal joint of their digit, the more tibial one coming into relation with the combined flexor profundus I and intermetatarsalis I. Over the third metatarsal the arrangement is more complicated. Proximally one finds two muscle bundles lying side by side and separated by a tendinous partition, but as one passes distally additional fibers, with a slightly different direction, become added upon either side, so that eventually four muscle bundles may be recognized over the bone (Fig. 6). The two lateral ones extend dorsally between the third and the adjacent metatarsals, while the two median ones, which are considerably smaller than the others, are confined to the plantar surface of the metatarsal. Eventually the plantar and lateral muscles of one side of the median line separate from the corresponding bundles of the other side, so that two pairs of muscles become recognizable which insert into the tibial and fibular metatarso-phalangeal sesamoid cartilages of the third digit. The two median bundles I take to be the flexores breves profundi III, while the lateral bundles are the intermetatarsales II and III.

In the case of the fourth digit a determination of the arrangement is somewhat difficult, but just as muscles pass from the hallux to the second digit, so muscles from the minimus pass to the fourth digit, and it will therefore be convenient to consider the two digits together. In sections taken near the bases of the metatarsals two indistinctly separated bundles are to be seen over the third intermetatarsal space and a third one lies

partly over the fourth space and partly over the tibial border of the fifth metatarsal. The two more tibial bundles, which probably represent but a single muscle, take their origin from the sheath of the peroneus longus tendon, while the fibular one seems rather to come from the base of the fifth metatarsal. Additional fibers are added to the fibular muscle from an origin on the tibial surface of the fifth metatarsal. These fibers are easily recognizable from their decidedly oblique direction, and it seems probable that they represent a distinct muscle, namely, the intermetatarsalis IV. Eventually the two more tibial bundles insert into the tibial metatarso-phalangeal sesamoid, while the fibular muscle, together with the intermetatarsalis IV terminates on the fibular sesamoid.

Over the plantar surface of the fifth metatarsal two muscles, which arise from the sheath of the peroneus longus tendon, occur. They are separated by a distinct tendinous partition, and, as they are traced distally, separate to be inserted into the two sesamoid cartilages of the metatarso-phalangeal joint.

Finally, I find beneath the hallucal slip of the flexor brevis medius str. superficiale and between that muscle and the conjoined flexor brevis profundus I and intermetatarsalis I a small bundle of muscle fibers, which may possibly represent an additional slip of the flexor brevis profundus layer. It is so rudimentary, however, that it is impossible to assign to it an origin or an insertion; it is distinctly separate from the combined intermetatarsal and flexor profundus I and its fibers have a direction almost at right angles to those of the flexor brevis medius str. superficiale above it. No equivalent of the muscle occurs either in the mouse or the cat, in both of which the hallux is considerably reduced as compared with that of the opossum.

In the mouse the arrangement of the flexor brevis profundus is very similar to that of the opossum, except that the various slips are much more intimately fused and consequently much more difficult to recognize. Over the base of the first metatarsal there is a mass of muscular tissue in which no indications of a composite nature could be detected. It passes to the tibial metatarso-phalangeal sesamoid of the second digit and seems therefore to correspond to the combined intermetatarsal and flexor profundus I of the opossum. Over the second metatarsal are two muscle slips separated only by a tendinous partition. They both insert into the fibular metatarso-phalangeal sesamoid of the digit, the arrangement differing in this respect from that of the opossum. The muscles of the third digit are arranged as in the opossum, though much more extensively fused. The mass which they form curves dorsally around each surface of the metatarsal and the lateral parts of the crescent so formed

are separated from the central portion by a tendinous partition. The entire mass eventually separates into two portions which insert respectively into the tibial and fibular metatarso-phalangeal sesamoids of the digit and seem to represent the two flexores breves profundi III together with the intermetatarsales II and III. In the fourth digit also there is much fusion of slips. A relatively large bundle passes from the fifth metatarsal to the fibular sesamoid of the digit, representing the fourth intermetatarsal, but whether it also includes a portion of the flexor profundus IV remains uncertain. The main mass of the flexor profundus V passes to the fibular sesamoid of its digit, but it gives off a slip which unites with the adductor slip for the digit and appears to represent the tibial portion of the flexor profundus V.

In the cat, owing to the reduction of the hallux, no representatives of the flexor brevis profundus I nor of the intermetatarsalis I exist. Otherwise the arrangement resembles closely that of the mouse. In the second digit only the two slips of the flexor brevis profundus (Fig. 7, *fbp*^{2}), occur, and these pass to the two metatarso-phalangeal sesamoids of the digit. Both slips of the flexor profundus V are well developed.

A comparison of the mammalian plantar muscles as described above with those of the lacertilia may be made as follows:

Lacertilia.	*Mammalia.*
Flexor brevis superficialis str. superficiale.	Flexor brevis superficialis str. superficiale. Abductor hallucis.
Abductor V.	Abductor ossis metatarsi V. Abductor V.
Flexor brevis superficialis str. profundum.	Flexor brevis superficialis str. profundum.
Flexor brevis medius str. superficiale.	Lumbricales. Hallucal slip of flexor brevis medius str. superficiale.
Flexor brevis medius str. profundum.	Adductors.
Flexor brevis profundus intermetatarsales.	Interossei.

IV. The Plantar Muscles in Man.

It remains now to consider the plantar muscles of the human foot in the light of the conclusions reached in the preceding pages, and in doing so the nomenclature employed in human anatomy may be followed.

The *flexor brevis digitorum* presents little difficulty. It is clearly homologous with the main mass of the flexor brevis superficialis str. superficiale, and certain of the peculiarities it presents are explicable on the basis of the phylogenetic history of that muscle. Thus the frequent origin of the slip for the minimus from the plantar surface of the tendon of the long flexor is clearly a reminiscence of the significance of the tendon as a portion of the plantar aponeurosis, and it merely obscures the true relationship to regard the slip having such an origin as something distinct from what is usually regarded as the normal slip.

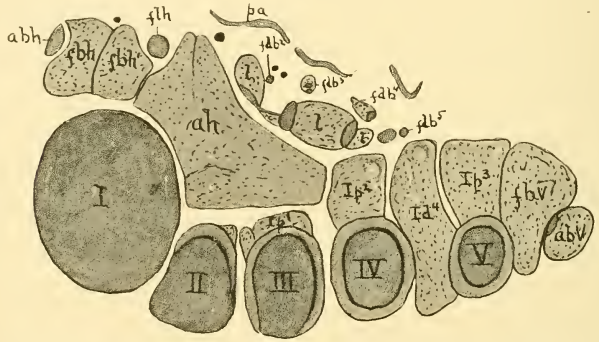


FIG. 8. Transverse section through the foot of a human fetus of 9 cm. *abh* = abductor hallucis; *abV* = abductor quinti digiti; *ah* = adductor hallucis; *fbh* and *fbh'* = medial and lateral heads of flexor brevis hallucis; *fbV* = flexor brevis quinti digiti; *fdb* = flexor brevis digitorum; *flh* = tendon of flexor longus hallucis; *Id* = dorsal interosseus; *Ip* = plantar interosseus; *l* = lumbricalis; *I-V* = metatarsal bones.

The stratum profundum of the flexor brevis superficialis is so evident a constituent of the plantar musculature both in the lacertilia and mammalia, that its occurrence in the human foot seemed more than likely. An examination of sections of a foot from a fetus of 9 cm. revealed what I take to be its representative. Before the flexor brevis begins to divide into its terminal slip, tendons appear imbedded in the center of the muscle mass, and of these, in the foot in question, there were four, notwithstanding the fact that the tendon for the fifth digit, as is so often the case, was derived from the tendon of the long flexor. The muscle fibers surrounding the most medial tendon gradually separated from the rest to form the slip to the second digit, and those sur-

rounding the most lateral tendon similarly separate to form the slip for the fourth digit. The fibers surrounding the two remaining tendons arrange themselves in two bundles, one lying immediately above the other, which, with their tendons, eventually unite to form the slip for the third digit (Fig. 8, *fdb* ^{3}). I take the more dorsal of the two bundles to represent the flexor brevis superficialis str. profundum; the slip to the third digit being the only portion of it persisting.

The flexor brevis digitorum has been taken to be the exact equivalent of the flexor sublimis digitorum of the arm, and on that basis has been regarded as primarily a crural muscle which has secondarily descended into the foot. If the homologies traced in the preceding pages be correct, there are no grounds for assuming a descent of the muscle from the crural region. It is from the beginning an intrinsic muscle of the foot.

The *abductor hallucis* (Fig. 8, *abh*), and *abductor quinti digiti* (*abV*) likewise require but brief consideration. There seems no doubt but that they are equivalents of the correspondingly named muscles in the lower mammals and are therefore derivatives of the primary superficial plantar layer.

In the case of the *flexor brevis hallucis*, however, the matter is more complicated. The conception of it as a single muscle, so constantly found in text-books, probably dates back to Albinus, **34**, by whom it was thus described, and is sufficiently satisfactory to the anatomist who studies muscles through physiological spectacles. So soon as a morphological basis is sought for the classification of muscles, this one loses its simplicity. Cruveilhier, **77**, reserves the name flexor hallucis brevis for the more medial portion of the muscle, regarding the lateral portion as a part of the adductor, and in this he has been followed by Flemming, **87**, and Gegenbaur, **92**, as well as by Ruge, **78a**, in his comparative studies. Cunningham, **82**, however, regards the two heads as representing the hallucal portions of his intermediate layer, the layer to which the plantar interossei belong and which, in typical cases consists of a pair of muscles for each digit.

From my own studies I am compelled to dissent from the interpretation which Cunningham gives to the muscle and to side with Cruveilhier and Flemming in regarding it as composed of two distinct portions which belong to different layers. My reasons for this belief are based largely upon the results obtained from the studies recorded in the preceding pages, which seem to point to the morphological independence of the two heads of the muscle. But confirmation of this view is

afforded by the frequency with which, throughout the mammalia, as described by Cunningham, 82, the lateral head disappears, leaving the medial head as the sole representative of the muscle, and also by the fact pointed out by Ruge, 78, and which I can confirm, that the tendon of the flexor longus hallucis throughout the greater part of its relationship with the muscle rests *upon* and not to the medial side of the lateral head (Fig. 8). For if the medial head, as there is every reason to believe, is a portion of the flexor brevis superficialis, then a muscle lying dorsal to the long flexor tendon cannot be regarded as belonging to the same layer that it does.

In accepting this view I differ, however, from Cruveilhier and Flemming as to the morphological significance of the lateral head. The medial head (Fig. 8, *fbh*), as just stated, is undoubtedly a portion of the flexor brevis superficialis, but instead of referring the lateral head to the adductor layer, *i. e.*, to the flexor brevis medius str. profundum I would assign it to the flexor brevis medius str. superficiale, *i. e.*, to the layer from which the lumbricals are derived. This conclusion is based upon the comparative series shown by the lacertilia, the opossum and man, in all of which the same muscle is recognizable and in the first named group is evidently a portion of the flexor brevis medius str. superficiale. The muscle, then, may be regarded as the lumbrical of the hallux.[1]

Flemming, 87, attaches considerable importance to the nerve supply of the lateral head being from the lateral plantar, while that of the medial head is from the medial plantar. In so describing the supply he follows the account given in most of the continental anatomies. Cunningham, 87, however, takes exception to this, stating that not only in the human foot but in those of all the mammals he studied, with one exception, the supply of both heads was from the medial plantar and my own observations on one adult and two fetal human feet give the same result. The nerve supply, however, cannot be taken as a criterion in this matter, especially if the lateral head of the muscle be regarded as belonging to the lumbrical layer; for the lumbrical of the second digit is normally supplied by the medial plantar and, furthermore,

[1] It seems probable that the corresponding medial head of the flexor brevis pollicis has the same significance. In an earlier paper, 03, I referred it to the adductor set, but the arrangement in the foot throws new light upon the question. Young, 79, has identified in the Rock kangaroo a muscle, distinct from the flexor brevis pollicis, as a pollical lumbrical, but its isolated occurrence makes it questionable whether it can properly be regarded as such, rather than as an anomaly.

Brooks', 87, observations on the innervation of the lumbricals show that a confusion of the constituents of the two plantar nerves may occur, similar to that which may obtain between the ulnar and median in the hand.

A word concerning the origin of the flexor brevis hallucis may not be out of place. In certain English text-books, Morris, 3d Ed., and Cunningham for example, it is stated to arise in part from the cuboid bone. This may possibly represent its physiological origin, but it certainly gives a very incorrect idea of its morphological relations. It is very clear from the study of fetal preparations that the muscle has its origin primarily from the first cuneiform and secondarily from a dense lamella of connective tissue which forms a sheath for the tendon of the flexor longus hallucis and proximally is continuous with a dense fascia covering the tendon of the peroneus longus and also with a strong ligament which extends from the navicular to the third cuneiform. It is through its connection with the peroneal sheath that the muscle reaches

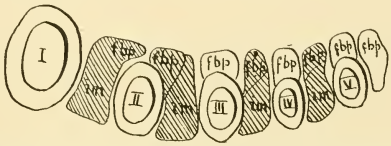


Fig. 9. Diagram to show the constitution of the interossei in the human foot. *fbp* = flexor brevis profundus; *im* = intermetatarsal; *I-V* = metatarsal bones.

the cuboid, but it is a mistake to suppose that this bone forms part of its true morphological origin.

The *lumbricales* are, as in the hand, clearly representatives of the flexor brevis medius stratum superficiale of the lower forms. And, similarly, both portions of the *adductor hallucis* are portions of the flexor brevis medius str. profundum, Ruge's, 78, observations on the development of the caput transversum, apart from comparative studies, showing its embryological relations to the caput obliquum. Meckel's, 32, identification of the caput transversum as a lumbrical is unsupported by either embryological or comparative evidence.

The *interossei* resemble closely those of the hand, whose phylogeny I have already, 03, considered. They represent a combination of the flexores breves profundi and the intermetatarsales, and their constitution may be understood from the accompanying diagram (Fig. 9). Considering first the three central digits; each possesses two slips of the flexor brevis profundus. Both slips of the second digit unite with

the first and second intermetatarsals forming the first and second dorsal interossei, which insert into the proximal phalanx of that digit. The medial slip of the third digit forms the first plantar interosseous, while the lateral slip unites with the third intermetatarsal to form the third dorsal interosseous; the medial slip of the fourth digit forms the second plantar interosseous, while its lateral slip unites with the fourth intermetatarsal to form the fourth dorsal interosseous.

In the fifth digit both slips of the flexor brevis profundus retain their separate individualities, the medial one forming the third plantar interosseous, while the lateral one is the muscle known as the *flexor brevis quinti digiti.*

I have found nothing in my preparations that I could regard as a hallucal flexor profundus, although on comparative grounds such muscles should be expected. There is a probability, judging from what occurs in the opossum, that the first dorsal interosseous contains an element representing a hallucal flexor profundus, just as is the case in the hand, but of this I have not been able to obtain definite evidence. The so-called interosseous primus volaris of Wood, 67, does not appear to belong to the interosseous set of muscles; its position towards the medial surface of the hallux and its relations with the abductor are opposed to such an assignment of it. It seems rather to be a slip of the flexor brevis hallucis which has retained its primary origin from the first cuneiform, instead of shifting to the plantar aponeuroses with the rest of the muscle. So too the portion of the oblique head of the adductor hallucis, which Henle, 71, regards as the equivalent of the interosseus primus volaris of the hand, is rather to be regarded as a portion of the flexor brevis medius str. profundum.

In the preparations I have studied there is no distinct *opponens hallucis,* nor is the *opponens quinti digiti* represented as a distinct muscle. I am unable to determine the significance of the former muscle, whether it be a derivative of the adductor or the flexor brevis hallucis, but viewing the possibilities as they appear in my preparations I am inclined to look to the adductor for its origin (cf. Brooks, 87). In a fetus of 9 cm. I find that a portion of the flexor brevis quinti digiti inserts upon the upper part of the fifth metatarsal, a fact which seems to point to the derivation of the opponens quinti digiti from the flexor brevis. In this opinion I am in accord with Ruge, 78. It is certainly a very different structure from the so-called opponens quinti digiti of the cat (see p. 426).

Many difficulties are encountered in the working out of the detailed

homologies of the human plantar muscles, and the final determination of some of them requires more extensive material than has been at my disposal. But one point is, I believe, conclusively settled, and that is the arrangement of the human plantar muscles in a series of layers which are homologous with those found in both the reptilia and the amphibia. This point established gives a broader basis for comparison than the study of individual muscles can afford, and, it is to be hoped, will lead to a full understanding of the morphology of the plantar muscles. The identifications described above may be represented in tabular form as follows:

Lacertilia.	*Man.*
Flexor brevis superficialis str. superficiale.	Flexor brevis digitorum. Medial head of flexor brevis hallucis. Abductor hallucis. Abductor quinti digiti.
Flexor brevis superficialis str. profundum.	Portion of slip of flexor brevis digitorum to 3d digit.
Flexor brevis medius str. superficiale.	Lateral head of flexor brevis hallucis. Lumbricales.
Flexor brevis medius str. profundum.	Adductor hallucis. Opponens hallucis?
Flexor brevis profundus. Intermetatarsales.	Flexor brevis quinti digiti. Opponens quinti digiti. Interossei.

Summary.

1. The plantar muscles in the urodele amphibia are arranged in four layers and are all intrinsic to the foot, arising either from the plantar aponeurosis or from the bones of the foot.

2. In the lacertilia the number of layers becomes increased to six by the division of the superficial and middle layers so that in each of them there is a stratum superficiale and a stratum profundum.

3. The plantar aponeurosis has also differentiated into two layers, the deeper of which forms the plantar portion of the tendons of the long flexors.

4. In the mammalia the six layers found in the lacertilia persist.

5. The marginal portions of the flexor brevis superficialis early separate from the main mass of the muscle and form the abductors of the hallux and minimus.

6. The mammalian flexor brevis hallucis is a compound muscle; its medial head is derived from the flexor brevis superficialis and its lateral head from the flexor brevis medius stratum superficiale.

7. The flexor brevis superficialis stratum profundum is represented in the human foot by a muscle bundle and tendon which unites with the slip of the flexor brevis digitorum passing to the third digit.

8. The flexor brevis digitorum is not a crural muscle which has secondarily descended into the planta. It is from the beginning an intrinsic muscle of the foot.

9. The flexor brevis quinti digiti is not equivalent to any portion of the flexor brevis hallucis, but is a portion of the flexor brevis profundus layer.

10. The oblique and transverse heads of the adductor pollicis are primarily parts of a single muscle and are portions of the flexor brevis medius stratum profundum.

11. The opponens hallucis is probably a derivative of the oblique head of the adductor hallucis; the opponens quinti digiti is a portion of the flexor brevis quinti digiti and therefore a portion of the flexor brevis profundus.

12. The dorsal interossei are formed by the fusion of portions of the flexor brevis profundus with the intermetatarsales, the plantar interossei being formed by the remaining portions of the flexor brevis profundus.

Anatomical Laboratory, University of Michigan,
January 11, 1907.

REFERENCES.

Albinus, B. S., 34.—Historia Musculorum Hominis. Leidæ Batavorum. 1734.

Baur, G., 85.—Zur Morphologie des Tarsus der Säugethiere. Morph. Jahrb., X, 1885.

Bischoff, T. L. W., 70.—Beiträge zur Anatomie des Hylobates leuciscus. Abh. math.-phys. Classe königl. Bayerischen Akad. Wiss., X, 1870.

Brooks, H. St. J., 87.—Variations in the nerve supply of the lumbrical muscles in the hand and foot, with some observations on the innervation of the perforating flexors. Journ. Anat. and Phys., XXI, 1887.

Brooks, H. St. J., 88.—On the short muscles of the pollex and hallux of the anthropoid apes with special reference to the opponens hallucis. Journ. Anat. and Phys., XXII, 1888.

Coues, E., 72.—The osteology and myology of Didelphys virginiana. Mem. Boston Soc. Nat. Hist., II, 1871-1878.

Cruveilhier, J., 77.—Traité de l'Anatomie. 3me Edn. Tome I, 1877.

Cunningham, D. J., 78.—The intrinsic muscles of the hand of the Thylacine (Thylacinus cynocephalus), Cuscus (Phalangista maculata) and Phascogale (Phascogale calura). Journ. Anat. and Phys., XII, 1878.

Cunningham, D. J., 78a.—The intrinsic muscles of the mammalian foot. Journ. Anat. and Phys., XIII, 1878.

Cunningham, D. J., 82.—Report on some points in the anatomy of the Thylacine (Thylacinus cynocephalus) Cuscus (Phalangista maculata) and Phascogale (Phascogale calura), etc. Scient. Results Voyage of H. M. S. Challenger. Zool., V, 1882.

Cunningham, D. J., 87.—The flexor brevis pollicis and the flexor brevis hallucis in man. Anat. Anzeiger, II, 1887.

Flemming, W., 87.—Ueber den flexor brevis pollicis und hallucis des Menschen. Anat. Anzeiger, II, 1887.

Gadow, H., 82.—Beiträge zur Morphologie der hinteren Extremität der Reptilien. Morph. Jahrb., VII, 1882.

Gegenbaur, C., 92.—Lehrbuch der Anatomie des Menschen. 5te Aufl. Bd. I, Leipzig, 1892.

Henle, J., 71.—Handbuch der systematischen Anatomie des Menschen. 2te Aufl. Bd. I, 1871.

Hoffmann, C. K., 90.—Reptilien in Bronn's Klassen und Ordnungen des Thierreichs. Bd. VI, Abth. III, 2. Leipzig, 1890.

Humphry, 72.—The muscles and nerves of Cryptobranchus Japonicus. Journ. Anat. and Phys., VI, 1872.

McMurrich, J. P., 03.—The phylogeny of the palmar musculature. Amer. Journ. Anat., II, 1903.

McMurrich, J. P., 04.—The phylogeny of the crural flexors. Amer. Journ. Anat., IV, 1904.

Meckel, J. F., 32.—Manual of general, descriptive and pathological anatomy. Translated from the French by H. Sidney Doane. Philadelphia, 1832.

Perrin, M. A., 93.—Contribution à l'étude de la myologie comparée: Membre postérieur chez un certain nombre de Batraciens et de Sauriens. Bull. Scient., XXIV, 1893.

Reighard, J., and Jennings, H. S., 01.—Anatomy of the Cat. New York, 1901.

Ruge, G., 78.—Entwicklungsvorgänge an der Muskulatur des menschlichen Füsses. Morph. Jahrb., IV, Suppl., 1878.

Ruge, G., 78a.—Zur vergleichenden Anatomie der tiefen Muskeln in der Fusssohle. Morph. Jahrb., IV, 1878.

Wood, J., 67.—Variations in human myology observed during the winter session of 1866-67 at King's College, London. Proc. Roy. Soc., London, XV, 1867.

Young, A. H., 79.—The intrinsic muscles of the marsupial hand. Journ. Anat. and Phys., XIV, 1879.

ON THE GROSS DEVELOPMENT AND VASCULARIZATION OF THE TESTIS.

BY

EBEN C. HILL.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 14 TEXT FIGURES.

While studying the development of the blood supply to the Wolffian bodies, my attention was called to the interesting manner in which the testis becomes vascularized. Accordingly, following a suggestion from Professor Mall, I injected a series of embryo pigs and later the testes of adult pigs, hoping that a thorough knowledge of the blood supply of this gland in the pig would facilitate the study of the vascularization of the human testis. In this I was mistaken, for the information gained from corrosions, injections, and cleared preparations of the testis of the pig was of comparatively little value in unravelling the interesting though complex blood supply of the sex gland in man. Hence, in this paper I shall confine myself to the gross development and blood supply of the pig testis, and will later publish the results of studies of the human gland.

The blood supply to the human male sex gland, to use a rather unique term for anatomical literature, is rational. It is much as one might expect, knowing the lobular arrangement. The vascularity of the pig testis, on the other hand, is quite unusual and it is difficult to imagine what causes could have produced such an unique arrangement.

Literature.—The literature on the vascularization of the testis is surprisingly meagre, considering the enormous bibliography which has accumulated on spermatogenesis and the descent of the testis. Kölliker, Mihalkovics, Bardeleben, Pflüger, Waldeyer, and many others have added much to our knowledge of these two subjects, but as yet comparatively little has been accomplished toward unravelling the blood supply. Kölliker traced the spermatic artery as it branched to supply the cord, epididymis and testis. He states that the blood vessels follow the trabeculæ of the sex gland after penetrating the albuginea near the

epididymis. He also describes a capillary network around the tubules. Astley Cooper has investigated a capillary plexus covering the internal surface of the tunica albuginea which he has termed the tunica vasculosa. But beyond this there is little to be found in the literature of the subject.

The vascular units which Professor Mall and his students have shown to be present in several organs and which they assume are to be found in all organs, have not as yet been demonstrated in the testis, and, indeed, aside from what has been cited above, nothing is known of the arterial or venous supply of the male sex gland. Dr. Mall has frequently demonstrated the presence of certain units of the blood system which may or may not be peculiar to the organ in which they are found and which correspond to the histological or structural unit of the organ. These units are composed of small branching blood vessels which pass into capillaries and the blood from which is collected into small veins. This theory of vascular units may be briefly summed up in the statement "similar blood supply to similar histological units." These vascular units have been proved to be present in the liver,[1] spleen,[2] and adrenal,[3] but in the testis of the pig I can make out no definite units. In man, however, the lobular arrangement is less complex, and results have been so encouraging that probably these units will be shortly discovered.

Methods and Material.—The necessity of clearly understanding the development of the circulatory system in the earliest embryonic stages in order to properly interpret the course of the blood stream in the adult organs has been frequently emphasized. Accordingly, in attempting this research frequent use has been made of embryonic material. Embryo pigs, many of which were alive when delivered at the laboratory, were injected and cleared, and, after tracing the development of the circulation in these stages, the investigations were completed with adult material. For adult human testes I am indebted to Professor MacCallum of the pathological laboratory. To Professor Brödel I also wish to express my appreciation for several valuable specimens of human embryonic testes as well as for his helpful suggestions in making the illustrations. For the courtesies of their laboratory I wish to thank Professors Wiedersheim and Keibel of Freiburg.

All injections of embryonic material were made with India ink. In

[1] Mall, F. P.: A Study of the Structural Unit of the Liver. Am. Jour. Anat., Vol. V, No. 3.

[2] Mall, F. P.: The Structure of the Spleen. Johns Hop. Hosp. Rep.

[3] Flint, J. M.: The Blood Vessels, Angiogenesis, Organogenesis, Reticulum and Histology of the Adrenal. Johns Hop. Hosp. Rept., Vol. IX.

the youngest stages, measuring from eighteen mm. to seventy-five mm., a hypodermic syringe with a fine needle forced the injection fluid into one of the umbilical arteries, and by watching the hind legs and head excellent results devoid of extravasation could be obtained. This mode of injection is particularly desirable in these early stages for it is not necessary to rupture the surrounding membranes and thus the embryo is protected against injury in handling. In larger embryos injections were made directly into the aorta by puncturing the left ventricle. Considerable pressure was necessary to overcome in the earlier stages the resistance resulting from the small lumen of the spermatic artery, and in larger embryos because of its remarkable tortuosity. On account of this pressure the Wolffian bodies were frequently doubly injected, the injection mass passing through the sinusoids and capillaries described by Minot into the veins. In nearly all such specimens the testes showed only an arterial and capillary injection. In this connection it may not be amiss to emphasize the advantages of India ink in all cases where a fluid is desired which will flow wherever the blood stream goes, and yet is resistant to the ordinary laboratory acids and to concentrated solutions of potassium or sodium hydroxide.

After the injection of each embryo, the right testis together with the Wolffian body, kidney, and aorta were removed and placed in ninety-five per cent alcohol for clearing, while the left sex gland with its appendages was prepared for sectioning. Of the various clearing procedures, the modified Schultze method[4] was found to give the most satisfactory results. This method is as follows:

After the injections have been completed all unnecessary tissues surrounding the parts under investigation are removed and the specimens are placed in ninety-five per cent alcohol. The removal of adventitial tissue is most important, though entire embryos may be successfully cleared if openings are made into the abdomen, thorax and cranium. In embryos ranging above one hundred and fifty mm. in size, it is still better to make sagittal sections of the hardened specimens and to clear in halves. The alcohol should be frequently changed and large quantities should be used. In order to obtain transparent specimens the tissues must be completely shrivelled before removal from the alcohol, and the length of time necessary to accomplish this result depends, of course, upon the size of the objects. For very small specimens at least

[4] Hill, E. C.: On the Schultze Clearing Method as Used in the Anatomical Laboratory of the Johns Hopkins University. Johns Hop. Hosp. Bull., Vol. XVII.

three days should be allowed, while for large objects the time should not be less than a week. Experiments with absolute alcohol in place of ninety-five per cent alcohol gave no better results, and its use is an unnecessary expense. The coagulation of the proteid occurs almost as quickly in one percentage as in the other.

After the specimens have been sufficiently shriveled they should be placed in one per cent potassium hydroxide. When a higher percentage is resorted to, so rapid is the action that the safety of the specimen is endangered, and it was the use of the strong solutions recommended by Schultze which caused the loss of much valuable material. In this weaker solution the tissues become transparent in from four to forty-eight hours, depending upon the size of the specimens. After sufficient clearing in this medium they should be transferred to twenty per cent glycerine, in which clearing continues and a certain amount of hardening occurs, rendering the tissues firm enough to permit of dissection. Should the specimens be as transparent as is desired, they may be removed from time to time to higher percentages of glycerine till at last they are permanently stored in pure glycerine. A certain amount of shrinkage is noted in some organs after an immersion in this fluid for a year or more, but when the specimens are studied immediately after being cleared the measurements are practically the same as in the fresh tissue. The shrinkage which some observers have noticed is probably due to transferring the specimens too rapidly to higher percentages of glycerine. After some experimenting we have found that embryos hardened in formalin can be cleared also, though in this case 10 per cent potassium hydroxide is essential and the specimens must remain in this solution for several weeks or months.

Blood pigment in some instances will not be entirely removed by this process alone and in organs, such as the kidney, transparency can sometimes only be obtained by a secondary treatment. After passing through the one per cent potassium hydroxide as outlined above and being placed in twenty per cent glycerine, the specimens containing the objectionable pigment are treated with equal parts of fifty per cent ammonium hydroxide and one per cent potassium hydroxide. In this solution there is comparatively little danger to the specimen on account of the hardening produced by the twenty per cent glycerine. Indeed, in cases where it is deemed advisable for any reason to stop the clearing action, or it is found to be more convenient to continue the process at some future time, the objects may be removed to this twenty per cent glycerine and retained in this medium until a more fitting time when much higher

percentages of the caustic solution may be resorted to without danger to the specimens. The specimens may be studied in glycerine or by a method devised by Bardeen may be mounted upon glass slides and placed in any desired position in jars of glycerine. The objects are removed from pure glycerine, wiped and quickly washed. They are then placed in a little thick gelatin solution and are laid upon a warm glass slide. As soon as the gelatin is hardened the specimens are returned to the pure glycerine without any danger of becoming loosened from the slide. The purity of the glycerine should be assured, as the presence of foreign substances such as water may tend to soften the gelatin.

The clearing reagents advocated by Van Wijhe[5] and Lundvall[6] did not give the same degree of transparency as was gained by following the above method, though in clearing the capsule of the adult testis beautiful specimens were obtained by using absolute alcohol and xylol as outlined by Van Wijhe. In following the distribution of the blood vessels in the capsule, quite satisfactory results were obtained by the very practical and simple method devised by Flint in his work on the adrenal.[3] "After carefully dissecting away all of the paricapsular connective tissue from the injected and hardened gland, it is cut in half, longitudinally, with a sharp razor and the parenchyma is scraped out with a scalpel. The remaining fibrous capsule is then treated exactly like a section and, after dehydration, is cleared and mounted in a cell."

Although the modified Schultze method is particularly applicable to embryonic tissues, yet sections of the adult testis 3-4 mm. thick, the arteries and veins of which had been injected with India ink were speedily rendered transparent by a clearing treatment similar to that for the less resistant embryonic tissue.

In preparing injections for corrosion specimens of larger embryonic and adult testes, great difficulty was experienced until it was discovered that seven per cent celloidin would flow quite readily through a medium-sized hypodermic needle. Thus in injecting the adult sex gland it was only necessary to find the point at which the spermatic artery reached the gland in order to avoid the difficulty met with in forcing the injection mass through the many coils of artery which lie in the cord of the pig near its attachment to the epididymis. The corrosion was accomplished with hydrochloric acid and pepsin, after which the specimens

[5] Van Wijhe, J. W.: A New Method for Demonstrating Cartilaginous Mikroskeletons. Kononklijke Akademie van Wetenschappen Te Amsterdam, 1902.

[6] Lundvall, H.: Ueber Demonstration Embryonaler Knorpelskelette. Anat. Anz., pp. 219-223, Band XXV.

were washed and placed permanently in glycerine. Because of the thick and very resistant albuginea a rapid corrosion was more easily obtained when the fresh gland was placed for an hour in concentrated hydrochloric acid, after which it was treated in the usual way with a ten per cent aquous solution of this acid for twenty-four hours, followed by the ordinary peptic digestion in the thermostat.

Gross Development of the Testis.—Keibel in his Normentafel for the pig gives the anlages and traces histologically the development of the organs, but gives no measurements of these organs.

TABLE SHOWING IN MILLIMETERS THE LENGTH OF THE BODY AS COMPARED TO THAT OF THE KIDNEY, WOLFFIAN BODY, AND SEX GLAND OF PIG EMBRYOS.

The measurements were made from vertex to breech, and include all of the embryos in each uterus. In case of asymmetric development of these glands in any embryo averages were made of the lengths of both organs.

	Vertex-Breech.	Kidney.	Wolffian Body.	Testis or Ovary.
Uterus 1	20	1.2	7.3	1.5
	21	1.2	7.3	1.5
	20	1.1	7.4	1.4
	23	1.2	7.2	1.5
	22	1.3	7.1	1.4
	21	1.0	7.3	1.5
	20	1.2	7.2	1.5
Uterus 2	28	2.5	9.0	1.7
	28	2.6	8.0	1.6
	29	2.5	8.5	1.7
	27	2.4	9.2	1.7
	28	3.0	7.0	1.7
	29	2.7	8.7	1.4
Uterus 3	31	3.9	9.2	2
	33	4.0	9.1	2
	33	3.8	9.0	2
	35	3.7	9.1	1.9
	33	4.1	8.9	2
Uterus 4	40	5.8	10.0	3.2
	41	5.9	10.0	3.2
	40	5.9	11.0	3.1
	42	5.9	11.0	3.1
	41	5.8	10.0	3.1
	43	6.0	11.2	3.2
	41	5.8	10.5	3.2
	42	5.9	11.3	3.2
	39	5.6	11.5	3.1
	41	5.9	10.0	3.0

	Vertex-Breech.	Kidney.	Wolffian Body.	Testis or Ovary.
Uterus 5	49	7.3	11.0	3.5
	49	7.8	10.5	3.2
	49	6.8	12.0	3.2
	48	8.0	10.0	3.5
	50	7.8	10.5	3.5
Uterus 6	68	11.7	11.8	4.0
	67	11.5	12.0	4.0
	68	11.5	11.4	4.0
	69	11.4	11.5	3.7
	67	11.2	11.2	3.8
	68	11.5	11.7	4.0
	67	11.6	11.4	3.8
Uterus 7	85.0	14.5	10.2	4.6
	84.6	14 7	10.0	4.4
	84.4	14.3	10.5	4.6
Uterus 8	94.3	15.7	9.2	4.8
	94.5	15.9	9.0	5.0
	94.3	15.6	9.0	5.1
	94.0	15.5	9.8	5.0
	94.4	15:7	8.7	5.0
	94.3	15.6	8.9	4.7
Uterus 9	120.0	18.0	7.0	5.0
	121.0	19.0	7.2	5.3
	120.0	19.0	7.5	5.0
	120.5	18.5	7.0	5.5
	120.8	18.2	7.0	5.2
			Epididymis	Testis
Uterus 10	15.5	23.5		
	15.0	22.8	7.0	5.8
	15.8	23.0	7.0	5.6
	15.0	24.0	7.3	5.5
Uterus 11	210.0	31.0	8.2	6.0
	211.0	30.0	8.0	6.3
	210.5	35.0		6.5

A study of the foregoing table shows little variation in the body lengths of the embryos in each uterus. In the cases of abnormal development of the kidney, it is interesting to note the corresponding size of the Wolffian body. That a balancing of function exists between these two glands is suggested by the fact that an embryo having an unusually large kidney development has correspondingly small Wolffian bodies.

The measurements were made regardless of whether the sex gland was male or female, although in embryos beyond thirty-three mm. in length this sex distinction is observable.

In the adult pig testis the measurements vary considerably. The average might be placed at sixty-five mm. long, forty-two mm. deep and thirty-seven mm. wide with an average weight of sixty-eight grammes. These results are of interest when compared with those obtained by Krause[7] for the human adult sex gland. His averages were thirty-seven mm. long, twenty-eight mm. deep and twenty-four mm. wide, with the weight falling fifteen and twenty-four and a half grammes.

Development of the Blood Supply.—Concerning the embryonic development of the testis much has been written and it seems useless to enter into a discussion of the histogenesis and descent of the gland. Among the more recent studies of these subjects the article by Allen[8] on the ovary and testis of mammals will be found to contain a comprehensive survey of the literature and in this monograph the author outlines minutely the growth of the testis of the pig. He, however, makes no mention of the blood supply.

The first macroscopic indication of the vascularization of the testis is found when the embryo pig is thirty-three mm. in length. At this time the sex gland is situated relatively lower on the mesial surface of the Wolffian body, which may to some extent account for the low level at which the spermatic artery arises from the aorta. Concerning the origin of this artery there has been some discussion as to whether at times it may arise from one of the lower Wolffian arteries. Of the seventy-five or more specimens ranging in length from twenty-five mm. to two hundred and twenty mm., only one was found in which the artery arose otherwise than from the aorta. In this exception the spermatic artery came from the most caudal Wolffian artery close to its origin from the aorta.

In the thirty-three mm. stage seen in Fig. 1 no convolution is apparent in the spermatic artery which courses ventral to the Wolffian arteries. In this figure, as well as in the two following ones, it was found advisable to lay back the Wolffian body from its normal position in order to more clearly demonstrate the vascular supply to these glands. The renal artery which penetrates the kidney when the embryo is twenty-eight mm. in length is quite prominent and a few glomeruli are seen

[7] Krause, W.: Zum Spiralsaum der Samenfaden. Biol. Cent., 1881.

[8] Allen, B. M.: Embryonic Development of Ovary and Testis of Mammals. Am. Jour. Anat., Vol. III, No. 2.

in the cleared specimen. As in the human embryo, rotation of the kidney occurs before the entrance of the blood supply, as has been shown by Pohlman.[9] My study of sections of pig embryos places the rotation of the kidneys in this genus between twelve and fifteen mm.[10] In the human embryo Pohlman has shown that the vascularization occurs between twenty-five and thirty mm., while in the pig I find this vascularization of the kidney at twenty-eight mm. The adrenal, which is depicted

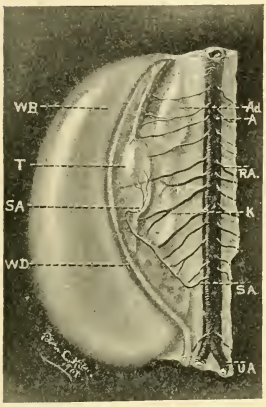


Fig. 1.

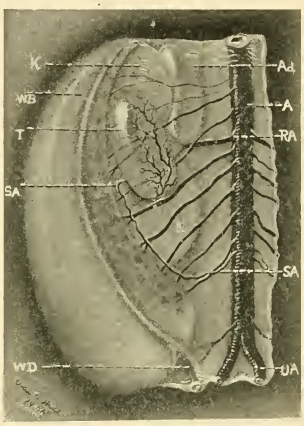


Fig. 2.

Fig. 1. Cleared specimen of the testis, kidney, and Wolffian body of an embryo pig 33 mm. in length, showing the first appearance of vascular supply to the testis. × 6. *W. B.*, right Wolffian body, 8.6 mm. in length; *Ad.*, adrenal; *A.*, aorta; *T.*, testis measuring 2 mm. in length; *S. A.*, spermatic artery; *K.*, kidney, 3.8 mm. in length; *W. D.*, Wolffian and Müllerian ducts; U. A., umbilical artery.

Fig. 2. Cleared specimen of the right testis, kidney, and Wolffian body of an embryo pig 48 mm. in length, showing the commencement of convolutions in the spermatic artery and the increased blood supply. × 6. *K.*, right kidney, 6.5 mm. in length; *Ad.*, adrenal; *W. B.*, Wolffian body, 10 mm. in length; *A.*, dorsal aorta; *R. A.*, renal artery; *T.*, testis, 3.5 mm. in length; *S. A.*, spermatic artery; *W. D.*, Wolffian and Müllerian ducts; *U. A.*, umbilical artery.

[9] Pohlman, A. G.: Concerning the Embryology of Kidney Anomalies. Am. Medicine, Vol. VII, No. 25.

[10] Hill, E. C.: On the First Appearance of the Renal Artery and the Relative Development of the Kidneys and Wolffian Bodies in Pig Embryos. Johns Hop. Hosp. Bull., Vol. XVI.

in the illustration merely as a land mark, is densely injected but no attempt has been made to show its blood supply and in this and the following series it appears as if uninjected.

The Wolffian body at this time receives from ten to twelve arteries which richly supply the gland. In many cases a pressure sufficient to insure a perfect injection of the sex gland resulted in a double injection of the Wolffian bodies and kidneys. In the Wolffian bodies the sinusoids described by Minot are beautifully demonstrated in sections of five to twenty μ in thickness.

Allen [8] has noted a great activity in the formation of the primitive sex cells of the seminiferous tubules and of the cords of Pflüger and rete cords about this time, and it is possible that this increased activity is due to the presence of the blood stream. Allen has also shown a sex differentiation in the embryo of twenty-five mm. which he bases upon histological observations. Through a study of the vascularization, this sex distinction is clearly marked at 33 mm., for as Clark [11] has shown "upon the peculiarities of each circulation the differential signs of sex are based, a visible dorsal vessel always indicating a male; an alabaster-like non-vascular white cortex a female embryo." This distinction, however, is true more particularly of the pig and is of doubtful value in differentiating the human sex glands.

In the embryo of forty-eight mm. (Fig. 2) the spermatic artery is found to have encircled a greater portion of the capsule of the sex gland and a certain amount of convolution is evident in the artery just before it reaches the testis. These convolutions are more marked as descent of the gland occurs, and this may be due in part to an attempt to shorten the artery. Thoma, however, in his studies of the development of the vascular system gives no such method of shortening. Nor, indeed, could this explanation account for the subsequent convolutions which occur after the testis has begun its descent from below the lower pole of the kidney. In this latter case there is a most decided lengthening accompanied by more marked convolutions. A similar condition is not found in the human embryo, nor to such marked extent in the mouse of this stage.

Microscopic sections demonstrate the capsular artery branching with a certain definite regularity on the surface of the gland, and sending minute arteries into the substance of the testis. A thick section shows these vessels entering perpendicularly and giving off branches which form capillary anastomoses around the medullary cords.

[11] Clark, J. G.: The Origin, Development, and Degeneration of the Blood Vessels of the Human Ovary. Johns Hop. Hosp. Rept., Vol. IX.

When the embryo has attained a length of eighty-seven mm. (Fig. 3) several of the anterior Wolffian arteries have disappeared and there is a decided atrophy of the organ itself. The capsular artery, a name which may be applied to that portion of the spermatic artery which supplies the albuginea and glandular substance proper, is seen to give off many

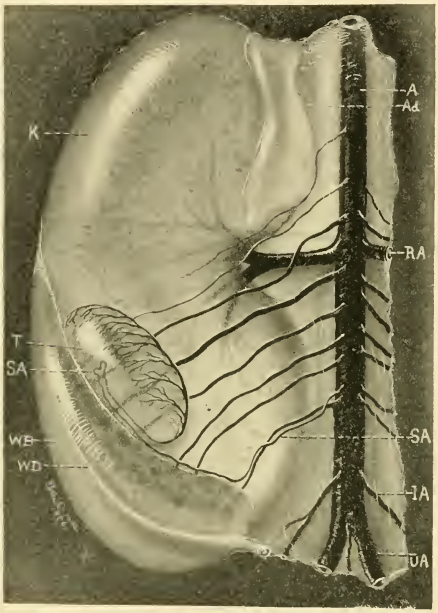


Fig. 3. Cleared specimen of the right testis, Wolffian body and kidney of an embryo pig 87 mm. long, showing descent of testis and atrophy of the anterior Wolffian arteries. × 6. *A.*, dorsal aorta; *K.*, right kidney, 14.7 mm. in length; *S. A.*, spermatic artery; *W. B.*, Wolffian body, 9.5 mm. in length; *W. D.*, Wolffian and Müllerian ducts; *I. A.*, iliac artery; *U. A.*, umbilical artery.

small branches, some of which are growing over the surface of the organ while others are penetrating deeply into the substance of the gland. In several specimens of this and later stages the capsular artery is found to divide into two main branches immediately after reaching the gland. The attachment of the sex gland to the Wolffian body is quite firm at this time.

Fig. 4 (128 mm.) shows a marked increase in the diameter of the spermatic artery, and also greater tortuosity of this vessel. The sex gland is seen to have assumed a different position in relation to the remains of the Wolffian body. This semi-rotation is, perhaps, caused by the convexity of the lower pole of the kidney as the testis in descending

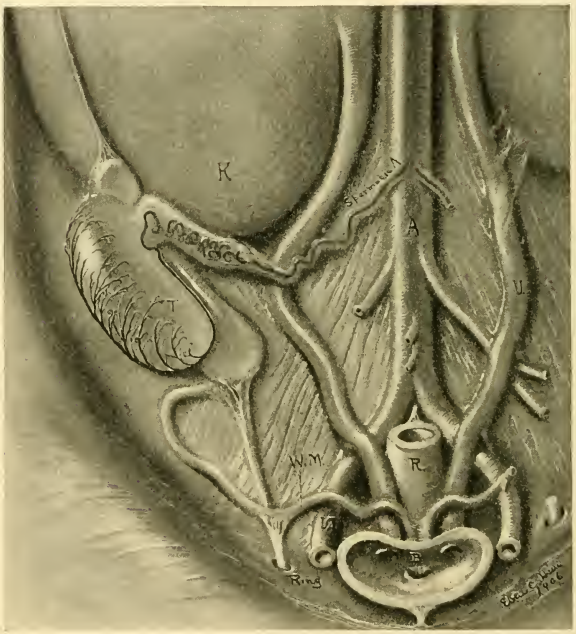


Fig. 4. Abdominal cavity of an embryo pig 128 mm. in length, cleared specimen of a right testis taken from an embryo of the same size and substituted in order to show the relative positions of the organs. × 6. This figure also shows the great tortuosity of the spermatic artery and by a comparison with Fig. 3, illustrates the occurrence of semi-rotation; *K.*, right kidney, 18.2 mm. in length; *S. A.*, spermatic artery; *A.*, dorsal aorta; *T.*, testis, 5.3 mm. in length; *U.*, ureter; *R.*, rectum; *W. M.*, Wolffian and Müllerian ducts; *U. A.*, umbilical artery; *B.*, bladder.

assumes a more dorsal position. Frequent anastomoses are noticed upon the capsule, and a small twig at the anterior end of the gland anastomoses with the branch from the spermatic artery which supplies the future globus major. Since the blood supply to the epididymis is not shown in any of the drawings, this branch has not been indicated.

The entrance of the testis into the internal ring occurs between the sizes of one hundred and ninety and two hundred and twenty mm. The left gland usually enters the internal ring first, and in Fig. 5 (210 mm.)

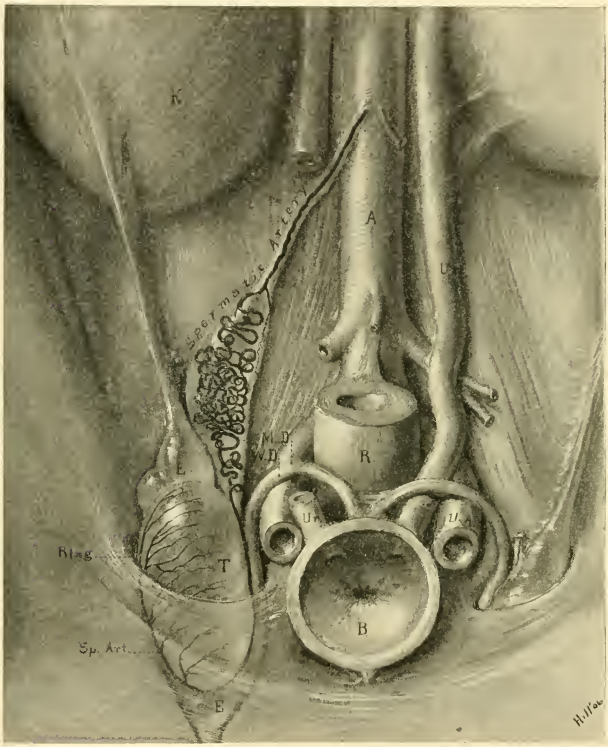


Fig. 5. A transparent specimen of the right testis of an embryo pig 210 mm. in length. The relation of this gland to the other organs was obtained from a fresh tissue specimen of an embryo of the same size. × 6. In this figure the left testis is seen to have nearly passed the internal ring, while the right sex gland has just entered. *K.*, right kidney; *A.*, dorsal aorta; *E.*, epididymis; *U.*, ureter; *R.*, rectum; *M. D.*, *W. D.*, Müllerian and Wolffian ducts; *U. A.*, umbilical artery; *B.*, bladder; *T.*, testis, 6 mm. in length.

only the globus major of the epididymis is apparent. The capsular artery has sent out branches which nearly encircle the sex gland, and

these encircling arteries have almost completed their growth around the testis. A certain limited portion lying close to the epididymis is never encroached upon by these branching arteries. From the spermatic artery before it reaches the testis several branches arise, from five to seven in number, which supply the cord and globus major and minor. Frequent anastomoses are seen on the albiginea, and small branches which encircle the anterior portion of the gland form anastomoses with

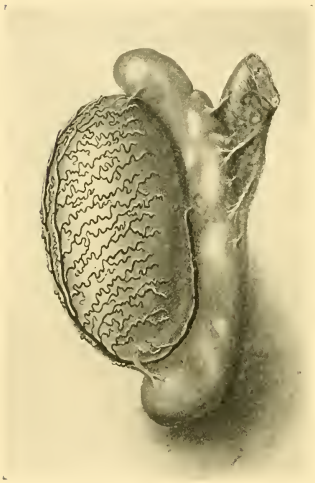


Fig. 6.

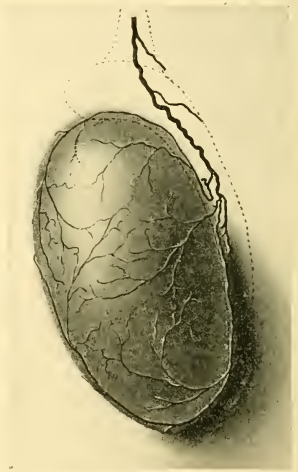


Fig. 7.

Fig. 6. The macroscopic appearance of an arterially injected adult pig testis, showing peculiar tortuous arrangement of the branches of the capsular artery in the tunica albuginea. × ⅔.

Fig. 7. A macroscopic drawing of the left injected testis of a human fœtus of seven months. × 6⅔. The dotted lines show the position of the cord and epididymis.

arteries supplying the globus major: thus allowing blood to penetrate the testis should the posterior portion of the capsular artery become occluded. The Müllerian ducts are atrophied and appear as ridges upon the Wolffian ducts which have increased in diameter of lumen and in thickness of wall.

The relations of the superficial blood supply in the testes of the adult pig and mouse and of the human fœtus of seven months together with

the comparative positions of the spermatic cords is shown in figures 6, 7 and 8. In the illustration showing the testis of the human embryo, the arteries are found to encircle the gland, being distributed to the under surface of the albuginea. Frequent anastomoses are formed similar in many ways to the adult superficial supply in the mouse, but differing materially from the arterial distribution in the capsule of the pig testis. The relative position of the spermatic cord to the epididymis is quite noticeable and may be due to the differences in the manner of suspension of the gland with reference to the horizontal plane of the body. The comparative size of the globus major and minor is also markedly different. In the pig the globus minor is at times as large and not infrequently larger than the globus major. Few convolutions are apparent in the human embryonic gland, while in the testis of the embryo mouse as well as in the adult a certain amount of tortuosity is met with, not, however, anywhere near as marked as in the pig.

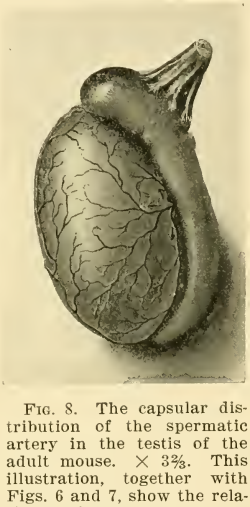


Fig. 8. The capsular distribution of the spermatic artery in the testis of the adult mouse. × 3⅔. This illustration, together with Figs. 6 and 7, show the relative positions of the cords and the albugineal blood supply.

Arterial and Venous Supply of the Adult Testis of the Pig.—The capsular artery gives off on the internal surface of the Tunica albuginea at rather regular intervals tortuous rib-like branches which nearly encircle the gland. These branches penetrate the substance of the gland following the septa and entering perpendicularly till they reach the mediastinum. Except in a very few instances no branches are given off from these perpendicular arteries until after the abrupt retro-flexion occurs near the center of the gland. After this sudden backward bending, many branches are given off which, coursing toward the surface of the testis, send off smaller twigs which in turn divide into capillaries around the seminiferous tubules and supply the stroma of the gland. The veins collect from these capillaries and merging into larger vessels follow the septa directly toward the albuginea where passing under the arteries on the internal surface of the tunica albuginea, they encircle the gland and passing toward the epididymis form the pampiniform

plexus. These veins are about twice the size of the branches from the capsular artery and show an intricate anastomosis. Upon cross section of a doubly injected gland some seven or eight perpendicular descending arteries will appear and perhaps eight to twelve collecting veins.

The extreme vascularity of the gland is shown in Fig. 10, which is an arterial and capillary injection made with India ink and cleared by

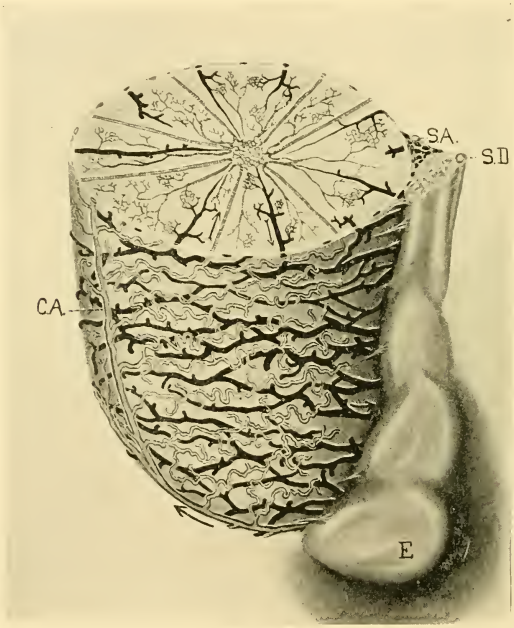


Fig. 9. A semi-diagrammatic representation of the circulation in the left testis of the adult pig. × 1⅔. *E.*, globus minor of the epididymis. The arrows indicate the course of the blood stream. The arteries and capillaries are red; the veins, blue.

the modified Schultze method. The anastomoses around the tubules are so profuse that they give the section an appearance of ancient mail armor.

A microscopic section of the injected testis, stained and cleared by the Van Wijhe method shows beautifully under low power the manner in

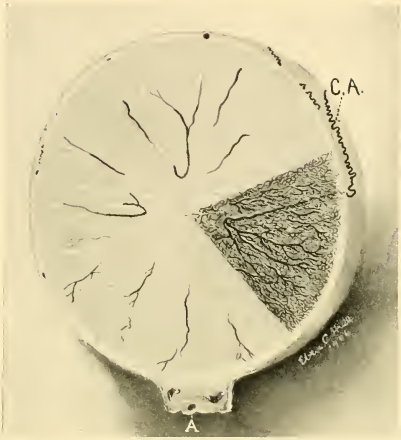


Fig. 10. A thick cleared cross section of the testis of an adult pig. × 1⅔. This shows the arteries and rich capillary network. *C. A.*, tortuous branch of the capsular artery which can be seen penetrating to the mediastinum of the gland and there forming the typical loop before giving off any branches. *A.*, spermatic artery in its course along the epididymis. The section was taken about midway between the globus major and minor, and as most of the epididymis was dissected away an atypically shaped piece of tissue remains.

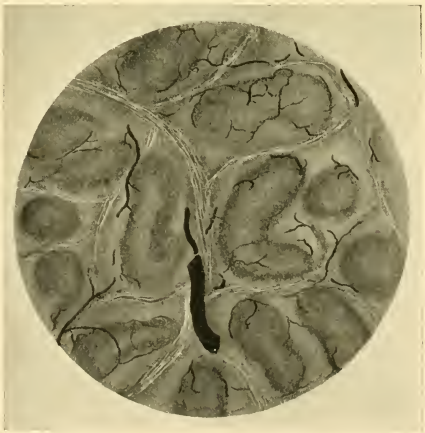


Fig. 11. A microscopic section of an injected testis of an adult pig cut 50 μ, showing the capillary supply around the tubules. × about 40.

which the capillaries encircle the tubules. In Fig. 11 are seen the capillaries, some larger arteries and a portion of one of the large ascending perpendicular branches given off shortly after the looping of the descending perpendicular branch near the center of the gland. The tubules of the pig testis show this capillary arrangement somewhat better than do those of the human adult male sex gland, for as was shown by Krause the tubules of the human testis measure two-tenths of a mm. in diameter, while I find that the tubules of the sex gland of the pig are between two-tenths and three-tenths mm. in diameter.

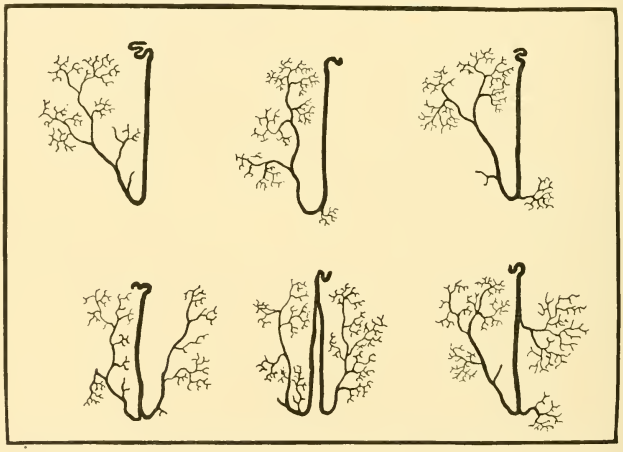


Fig. 12. Corrosion specimen of the testis of an adult pig, showing the typical arterial loops. × 2.

The various types of arterial loops comprising the descending perpendicular artery and its ascending branches are shown in Fig. 12. These loops were taken from corrosion specimens of the adult testis of the pig. In the figure they are arranged in order of frequency, the last having a recurrent branch before the abrupt looping, being very rare. No similar arrangement was found from studies of corrosions of the human gland.

What the causes are which produce this peculiar arrangement it is difficult to say. In the first five figures representing early embryonic stages the arteries were found to penetrate the gland perpendicularly but to give off branches as they descended.

This is depicted in Fig. 13.

No typical looping occurs till after the gland is in the scrotum and the subsequent rapid development has begun. This leads one to surmise that the sudden growth, which changes the embryonic organ from one measuring, 6 mm. x 3 mm. x 2.8 mm. unto the adult gland measuring 65 mm. x 42 mm. x 37 mm., is accountable for this peculiarity of blood supply. This seems to be especially plausible when the relative positions of the mediastina of the human and pig testes are compared. Probably the development is so rapid that the arteries which enter perpendicularly

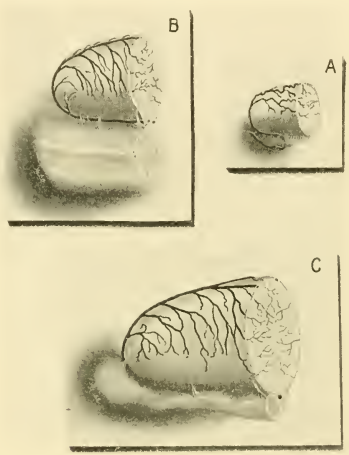


Fig. 13. *Fig. 13a* shows the left testis of an embryo pig 48 mm. in length. Then entrance of the perpendicular branches of the capsular artery is shown. × 9.

Fig. 13b illustrates the depth of penetration of these same arteries in the testis of an embryo pig of 87 mm. × 9.

Fig. *13c* shows the entrance and distribution of these same arteries in the left testis of an embryo pig of 210 mm. × 9.

in order to penetrate to the center are of necessity twisted back upon themselves in supplying the rapidly growing tubules whose development must be toward the circumference. The mediastinum is in the center of the gland and hence the tubules in developing radiate from this as a center.

The Blood Supply to the Albuginea—The vascular supply to the albuginea is shown in Fig. 14.

Above the large capsular arterial branches and veins which supply the

glandular tissue of the testis and which lie on the inner side of the tunica albuginea, is found a delicate plexus of small arteries, capillaries and veins grouped in irregular polyhedral forms, mostly of four or five sides, having an area of from nine to sixteen square millimeters. The arteries enclosing these polyhedrons spring from the encircling capsular branches and lie external to them. These small vessels are usually ac-

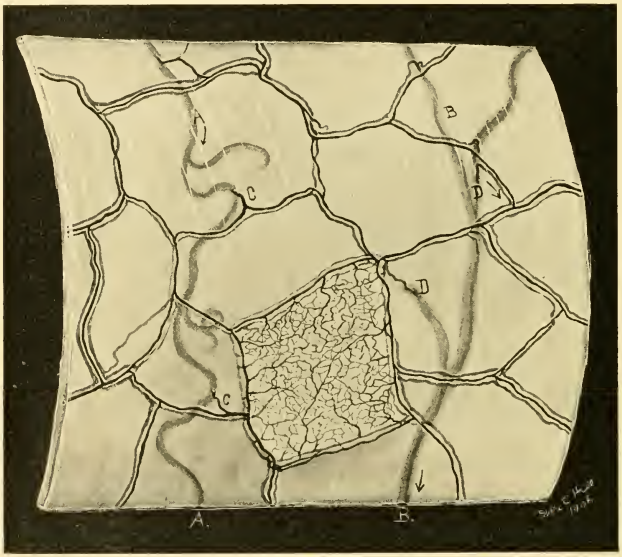


Fig. 14. A cleared specimen of the tunica albuginea of the testis of an adult pig, showing the arrangement of the vessels supplying this tunic. × 9. This specimen was studied in glycerine with a magnification of 80 diameters (Leitz) for the capillaries of the individual lobules, and with a magnification of 8 diameters for the arrangement of the lobules. Drawn with camera lucida. A., one of the tortuous capsular arteries given off from the main capsular artery. These vessels lie below the small arteries supplying the albuginea. *B*, an encircling capsular vein which ultimately empties into the pampiniform plexus. These veins also lie beneath the arteries and veins supplying the albuginea and also pass under the large capsular arteries. *C*, a small artery arising from a capsular artery. *D*, a small vein emptying into a capsular vein. The arrows indicate the course of the blood stream.

companied by venæ comites which carry back the blood to the encircling veins. Each lobule is filled with a network of capillaries. In corrosion

specimens this superficial albugineal blood supply appears as a fine mesh over the larger encircling arteries and veins. A few of the veins collecting from these superficial vascular lobules empty directly into the pampiniform plexus, but as a rule the course is as described.

Summary.

1. The measurements of the embryonic testis, Wolffian body and kidney from the time that the embryo is 20 mm. in length till the sex gland enters the internal ring are given.

2. A comparison of the size and weight of the human testis with that of the adult testis of the pig is made. The comparative sizes of the semniferous tubules of the adult pig testis and human testis is noted.

3. The testis of the pig receives its first blood supply when the embryo is 33 mm. in length, the kidney having received its blood supply when the embryo has attained a length of 28 mm.

4. Out of seventy-five specimens only one exception was found to the usual source of the spermatic artery, and in this case the artery instead of coming directly from the aorta arose as a branch from the most caudal Wolffian artery.

5. Marked convolutions in the spermatic artery are first evident when the embryo is 48 mm. in length.

6. A change in the position of the testis relative to the remains of the Wolffian body is noted between 110 and 130 mm. This change is almost a semi-rotation; the testis assuming a more lateral position and having the future epididymis between it and the aorta.

7. The entrance of the testes into the internal rings occurs when the embryo has attained a size of 190-220 mm. Generally the left testis enters first.

8. The differences between the superficial blood supply in the human embryonic testis and the testes of the adult pig and mouse are indicated, and the relative positions of the spermatic cords to the epididymes are shown.

9. The vascularization of the testis of the adult pig is diagrammatically represented, and a theory to explain the peculiarities of the arrangement of the vessels is advanced. This hypothesis is based upon a suggestion from Dr. Mall that the sudden growth of the testis brings about a backward looping of the arteries in order to supply the rapidly developing semi-inferous tubules.

10. The vascularization of the tunica albuginea is illustrated by a drawing made with the aid of a camera lucida.

EXPERIMENTAL EVIDENCE IN SUPPORT OF THE THEORY OF OUTGROWTH OF THE AXIS CYLINDER.[1]

BY

WARREN HARMON LEWIS.

From the Anatomical Laboratory, Johns Hopkins University.

WITH 21 FIGURES.

INTRODUCTION.

The controversy over the origin and development of the peripheral nerves has of late years assumed considerable importance, in that vigorous attacks have been made on the neurone doctrine as formulated by His, who considered the axis cylinder to be an outgrowth of the nerve cell.

Harrison[2] has most successfully controverted the cell chain theory, as advocated by Dohrn, and the Hensen theory of an early invisible connection between center and periphery, by a brilliant series of experiments on the larvæ of frogs, where he proves the axis cylinder to be an outgrowth of the nerve cell.

My experiments given here were not primarily directed towards the solution of the outgrowth theory, but I think offer, nevertheless, important evidence in support of this theory.

In transplanting the optic vesicle for the purposes of studying the origin of the lens and the development of the eye it often happened that a portion of the brain-tube adjoining the optic vesicle was transplanted with it. In experiment Pc_1, for example, the optic vesicle and an adjoining portion of the neural tube of an amblystoma embryo at a stage shortly after fusion of the neural folds was transplanted into the region between the optic vesicle and the medulla of another and slightly older amblystoma. The transplanted eye and brain tissue have con-

[1] The substance of this work was presented to the Association of American Anatomists December 28, 1905, and a brief summary is to be found in the Proceedings of the Association. Am. Jour. of Anat., Vol. V, No. 2.

[2] Further experiments on the development of peripheral nerves. Am. Jour. of Anat., Vol. V.

tinued their growth and differentiation in this strange location. From the small piece of brain there extend into the mesenchyme large numbers of nerve fibers (Figs. 1 and 2). These fibers extend in various directions into the mesenchyme without reaching end organs of any kind. For the most part the fibers are free from sheath cells. At first, of course, no protoplasmic bridges existed between the nerve cells in the transplanted brain, and any of the end organs of the host, and it is very difficult to imagine how they could have subsequently been formed. It is, of course, equally difficult to form a rational conception of how such bridges are subsequently transformed into nerves which take such an erratic course as those shown in Figs. 1 and 2. What possible end organs are there to account for this enormously greater number of nerves than are normally present in this region. Then again, these nerves are ones which, if the piece of brain had remained in its original position as a part of the brain, would, for the most part at least, have remained within the brain, and therefore the cells of the transplanted piece are ones which normally never connect in a direct way with peripheral end organs. I see no possibility of explaining these fibers by the Hensen doctrine. The absence of sheath cells makes it likewise impossible to explain their origin by the cell chain theory. It is evident also from this experiment that it is not necessary to have a predetermined path laid down before a nerve can grow out from its center towards the periphery. That there are such paths, and that they are necessary under normal conditions for the proper connection of the central nervous system with its end organs is in no way controverted by this experiment.

In experiment (IV_1) (Fig. 3) there is a somewhat similar piece of transplanted brain tissue connected with a transplanted optic vesicle of rana palustris. The section shows the differentiated piece of brain tissue ventral to the otic capsule and from it a large nerve has grown to the myotome. The piece was transplanted from the eye region into the otic region of the same embryo, whose neural folds had just closed, and so long before the nerves were present, and this nerve, as in the preceding experiment, occupies a strange path in the mesenchyme, and its presence can scarcely be explained in any other manner than as an outgrowth from the piece of transplanted brain. Whether the myotome has acted as a chemotactic agent or not can scarcely be determined from this one experiment alone, as it might be that the nerve has merely followed the path of least resistance which happened to be towards the myotome. Nor am I able to determine as to whether the nerve ends bear the same functional and anatomical relation to the muscle fibers that a normal motor nerve would.

In experiment DL_{38} (Fig. 4) there is another such piece of brain tissue connected with the transplanted eye, and between this piece of transplanted brain tissue and the irregular medulla is a large, strange nerve in a strange path. Its origin is certainly most satisfactorily explained by the outgrowth theory.

In another experiment (t_5) a small piece of the ectoderm from the region, destined to form medullar plate in a gastrula of rana palustris, was transplanted into the region ventral to the otic vesicle of another and older embryo of rana palustris. This piece of transplanted ectoderm has differentiated into nervous tissue and has sent out a long, large nerve, which passes through the mesenchyme into the region between the roof of the pharynx and the base of the cranial cartilage, where it ends abruptly in the mesenchyme (Fig. 5). This experiment illustrates at once the great power of self-differentiation various portions of the central nervous system possess, even when they are isolated at such very early stages. It is impossible to determine whether this nerve is an extra one or represents a nerve, which, if the piece of central nervous system had remained in its normal position, would have arisen from it and taken a normal course as a cranial or spinal nerve. It occupies a path which is in no sense predetermined and can only be explained by its having grown out from this piece of transplanted tissue probably along the path of least resistance or in a direction according to the orientations of the nerve cells from which it arose.

In experiment t_9 (Figs. 6 and 7) a piece of tissue just anterior to the dorsal lip of the blastopore of an embryo of rana palustris younger than that used in the preceding experiment was transplanted into an older embryo of rana palustris, whose neural folds had just closed. The piece of tissue lies in intimate contact with the dorsal wall of the pharynx in the region ventral to the otic capsule. It has differentiated into nervous system, and from it arises a nerve which runs in among the epithelial cells of the pharynx wall, divides into several bundles and can be traced for about 100 micro mm. and then appears to end in among these cells. This nerve clearly occupies a path in no sense predetermined for any known nerve, and the utter inadequacy any such explanation of its origin as would be given by a supporter of the Hensen doctrine is almost self-evident.

In another similar experiment (t_{10}) almost exactly the same conditions are to be found, where nerve fibers extend, from a piece of brain tissue in contact with the pharynx wall, into the wall itself among the epithelial cells.

Figs. 8, 9, and 10 are from embryos where the brain anterior to

the place of attachment of the eye was injured at the time of extirpation of the optic vesicle. These injuries have in some way resulted in irregularities in the brain in this region, that have given rise to extra nerves. These nerves extend from a region of the brain which, under normal conditions, never gives rise to peripheral nerves, or at least to nerves that leave the brain in the region to run into the mesenchyme. These nerves are, of course, unilateral and follow strange paths in the mesenchyme. The injury in the brain in these experiments was done long before the nerves normally appear. The nerves were not dragged out from the brain by the needle. The injury in some way made a path perhaps and may even have altered the orientation of some of the nerve cells.

In experiment bx_4 (Fig. 11) the fore part of the brain was removed before there were any traces of nerve fibers, without injury to the eyes or nasal pits. From the latter have arisen the olfactory nerves, which extend in various directions in the mesenchyme without reaching the brain. The nerve fibers are evidently outgrowths of the cells in the olfactory organ and are in no way connected with a central organ. It would seem impossible to explain their presence by the Hensen doctrine. In like manner the nerve fibers arise from a transplanted nasal pit. Fig. 12 is from a nasal pit which was transplanted beneath the ectoderm dorsal to the eye. After transplantation differentiation of the organ continued and nerve fibers were sent off into the region between it and the ectoderm, Fig. 13, but extend only a short distance.

The extraordinary interesting behavior of the optic nerve in the transplanted eyes afford very striking evidence of the outgrowth of the optic nerve from the eye.

The optic vesicle in these experiments was cut off and transplanted into the otic region of the same embryo, shortly after closure of the neural folds and long before the optic nerve forms.

In the majority of transplanted eyes the optic nerve pierces the retina as far as the outer or pigment layer, it then takes a course in among the pigment cells, often running for long distances as a compact bundle between these cells, and finally ending among them without entering the mesenchyme. Such nerves do not seem to be provided with sheath cells. Occasionally the optic nerve passes through the pigment layer into the mesenchyme and after running in it a short distance ends there.

In one embryo the optic nerve was evidently turned from its course in the outer molecular layer, in which layer it can be followed for many sections, and ends there without reaching even the pigment layer. Some-

times, in the somewhat irregular transplanted eyes, the optic nerve may divide as it passes through the retina, one branch passing out into the mesenchyme and the other entering the pigment layer.

Fig. 14, from experiment DF_7, shows a very remarkable condition, in which the transplanted eye touches the medulla in the region of the choroidal fissure. The optic nerve passes into the medulla. Fig. 15 shows an even more remarkable example of the course of an optic nerve from the eye to the medulla. The position of the choroidal fissure is on the ventral side of the transplanted eye and some distance from where the eye nearly touches the medulla. The nerve leaves the eye at the fissure, passes at first in among the cells of the outer layer (Fig. 16) then comes to lie close against the outer layer (Figs. 17 and 18), and finally jumps across a thin layer of mesenchyme into the medulla (Fig. 19). In some instances, the optic nerve after entering the medulla, can be followed as a fairly compact bundle of fibers for a considerable distance towards the anterior end of the brain, but in most of the specimens it soon disappears after entering the brain.

In a few of these somewhat irregular transplanted eyes the optic nerve takes a very curious course passing across the cup cavity from the ganglionic layer through the pupil and then into the mesenchyme (see Figs. 20 and 21), ending there. In both of these experiments a small bundle of optic nerve fibers pierces the retina as far as the pigment layer. In transplanting these eyes the ganglionic layer was probably injured in such a way as to interfere with the normal path of the nerve fibers, and so they have probably followed the path of least resistance through the pupil and out into the mesenchyme.

It would seem to me impossible to explain these various conditions of the optic nerve on any other basis than that they are outgrowths of nerve cells of the ganglionic layer of the retina.

These experiments furnish important evidence in favor of the outgrowth theory of the nerve fiber, and also that the fiber is a process of a nerve cell and not from a chain of cells.

Nerves do not necessarily need predetermined paths, but may grow into the mesenchyme in various directions, probably along the path of least resistance. That under normal conditions normal nerves do follow predetermined paths is in no way disproved by the experiments.

The hap-hazard manner in which these various nerves grow into the mesenchyme without reaching any end organ or even apparently going towards any especial end organ, would indicate nerve fibers can grow out in the embryonic mesenchyme without being attracted there by a special chemotactic agent.

Fig. 1. Experiment Pc_1. Embryo amblystoma killed 21 days after operation. The optic vesicle and piece of brain tissue adjoining it were transplanted from an amblystoma embryo, of a stage shortly after fusion of the neural folds, before the nerves appear, into the region between the otic vesicle and medulla of an older embryo. Section through transplanted eye and brain showing large groups of nerve-fibers running out into the mesenchyme in various directions; most of them are without sheath cells. Portions of these fibers from the neighboring sections were projected into the figure. × 90 diameters.

Fig. 2. Experiment Pc_1. Section through another portion of this transplanted brain showing other groups of nerve-fibers leaving it. These various nerve-fibers have been projected into the figure from the neighboring sections. They do not include those in Fig. 1. × 90 diameters.

Fig. 3. Experiment IV_1. Embryo rana palustris killed 5 days after transplantation of right optic vesicle and part of adjoining brain wall to the region ventral and posterior to the otic capsule. Section through portion of transplanted brain, posterior end of otic vesicle and medulla. From the transplanted brain a large nerve passes directly through the mesenchyme to the myotome. The distal ⅕ of the nerve is projected into the figure from the adjoining sections. × 90 diameters.

Fig. 4. Experiment DL_{53}. Embryo rana sylvatica killed 5 days after transplantation of optic vesicle caudal to otic capsule. The ectoderm and medulla were injured during the operation in the region into which the eye was transplanted. Section through this injured region caudal to otic capsule and anterior to transplanted eye. There is an increase in the size of the irregular medulla at the place of injury, and from here a large extra nerve passes to the ganglionic mass connected with the injured place on the ectoderm. Ventral to this extra nerve is seen a portion of the 10th cranial nerve which joins the medulla here. × 90 diameters.

Fig. 5. Experiment t_5. Embryo rana palustris killed 12 days after having had a small piece of the medullary plate region from the gastrula of another rana palustris embryo, transplanted into the region ventral to the otic capsule. Section through this transplanted piece which has developed into brain tissue and sent off a long nerve which passes medianwards between the base of the cranial cartilage and the roof of the pharynx. This nerve ends in the mesenchyme. The ventral part of the otic vesicle is seen in the section × 90 diameters.

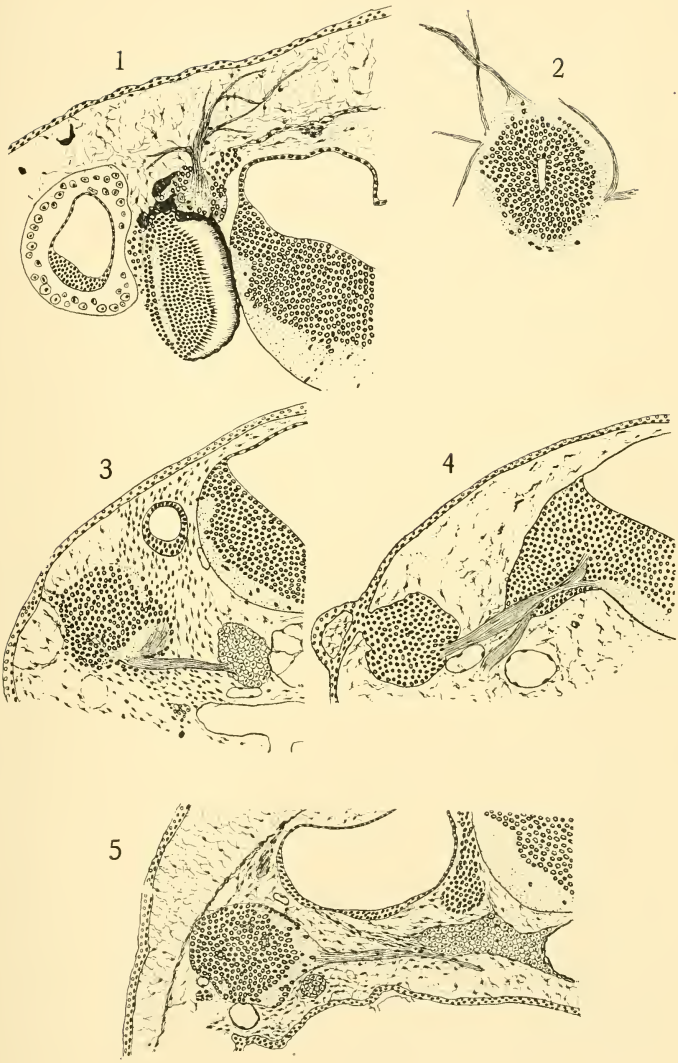

1
2
3
4
5

Fig. 6. Experiment t_9. Embryo rana palustris killed 12 days after having had a small piece of the ectoderm anterior to the dorsal lip of blastopore of an embryo of the same species transplanted into the otic region. The embryo at the time the piece was transplanted into it had completed the closure of the neural folds. An examination of the sections show that the transplanted piece has differentiated into nervous tissue like that of the central nervous system. The figure is from a section through this transplanted piece and shows its location ventral to the otic vesicle and in intimate contact with the dorsal wall of the pharynx. From this piece of brain tissue nerve-fibers pass into the epithelial wall of the pharynx and can then be traced in among the cells for about 100 μ. $\times$ 90 diameters.

Fig. 7. Experiment t_9. Section through pharynx wall showing position of nerve-fibers between the epithelial cells. $\times$ 360 diameters.

Fig. 8. Experiment DL_{68}. Embryo rana sylvatica killed 5 days after partial extirpation of the right optic vesicle. The brain anterior to the attachment of the optic stalk was injured. Section through injured region showing extra nerve which runs out into mesenchyme, it divides part, forms a loop, and enters the brain again dorsal to its origin; the main branch turns abruptly caudalwards and can be traced through many sections until it appears to join a portion of the Gasserian ganglion. A few sections caudal to the origin of this nerve another extra nerve arises from a similar position on the brain; it can only be traced a few sections out into the mesenchyme. The nerve shown in the figure from a composite of the 3 or 4 neighboring sections. $\times$ 90 diameters.

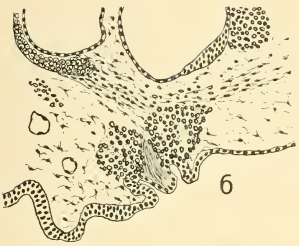
Fig. 9. Experiment DL_{48}. Embryo rana sylvatica killed 5 days after partial extirpation of the right eye. Section through anterior end of regenerated right eye and brain showing a small extra nerve running from the brain to a small ganglionic mass. This section is 80 μ anterior to the optic nerve, and on the normal side there is no trace of such a nerve between the olfactory and optic nerves. $\times$ 90 diameters.

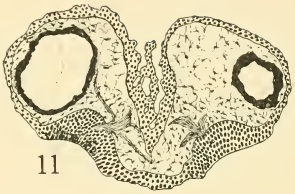
Fig. 10. Experiment DL_{68}. Embryo rana sylvatica killed 3 days after partial extirpation of the right eye. Section through fore-brain 100 μ anterior to optic nerve showing large extra nerve leaving brain and running out into mesenchyme. This nerve splits into several branches, the longest and largest runs caudally over the dorsal surface of the regenerated eye and is lost in the mesenchyme caudal to the eye. There is no corresponding nerve on the normal side. The brain on the right side is irregular and evidently was injured during the operation. $\times$ 90 diameters.

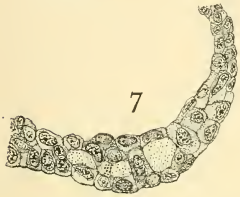
Fig. 11. Experiment bx_4. Embryo of amblystoma killed 9 days after removal of anterior portion of brain. The brain was removed through a dorsal incision before the first appearance of the cranial nerves. Section through anterior end of head showing anterior ends of eyes and nasal pits with nerve-fibers passing out into the mesenchyme from the latter. The ectoderm in the mid dorsal region dips down into the mesenchyme in the region from which the brain was removed. $\times$ 45 diameters.

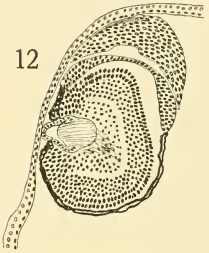
Fig. 12. Experiment Sp_1. Embryo of amblystoma killed 16 days after having had the nasal pit of another embryo transplanted beneath the ectoderm in the region dorsal to the right eye. Section through right eye and transplanted nasal pit. Where the nasal pit is in contact with the outer layer of the optic cup this portion of the outer layer is free from pigment and in places of greater thickness than normal. Between the nasal pit and ectoderm are olfactory nerve-fibers. $\times$ 45 diameters.

Fig. 13. Experiment Sp_1. Section through edge of nasal pit and ectoderm showing the position of the olfactory nerve-fibers. Sections anterior to this one show olfactory nerve-fibers passing from the nasal pit a short distance ventrally close to the median surface of the eye. $\times$ 180 diameters.

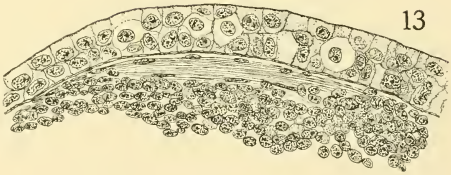

6

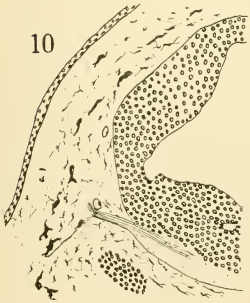

11


7


12


8


13


10

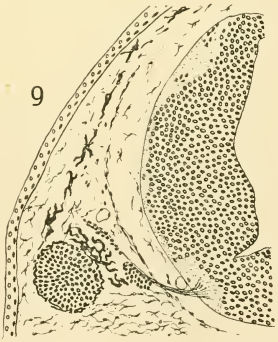

9

Fig. 14. Experiment DF_7. Embryo rana palustris killed 4 days after transplantation of optic vesicle into position caudal to normal eye region. Section through transplanted eye showing formation of optic cup and course of optic nerve from transplanted eye into medulla in the region of the V nerve. The optic nerve passes through the marginal veil into the grey matter where the fibers become scattered and can only be followed for a few sections anteriorly. The transplanted eye is separated from the ectoderm by mesenchyme and is without a lens. It is in contact with the medulla. × 90 diameters.

Fig. 15. Experiment DF_{17}. Embryo of rana palustris killed 5 days after transplantation of the optic vesicle into the region caudo-central to the otic capsule. Section through anterior end of transplanted eye showing position of optic nerve-fibers. The optic nerve is projected into this section in part from the adjoining section. × 90 diameters.

Fig. 16. Experiment DF_{17}. Section 30 micro mm. caudal to one in figure, showing optic nerve-fibers leaving retina and running among the cells of the outer layer. × 360 diameters.

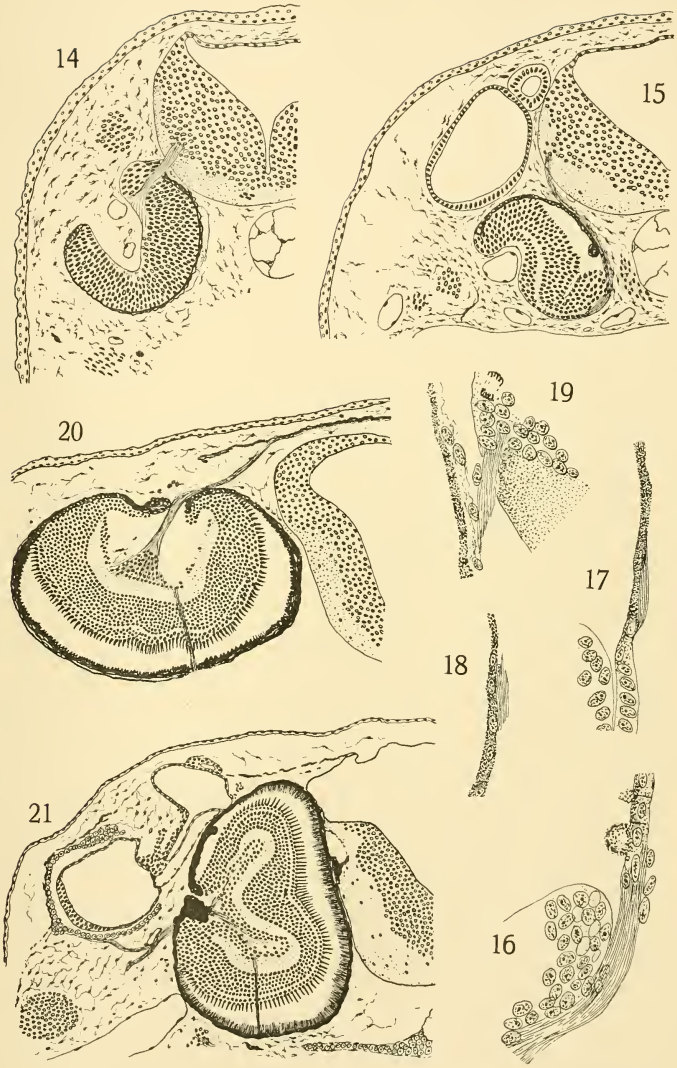
Fig. 17. Experiment DF_{17}. Section 50 micro mm. caudal to above, showing optic nerve-fibers close against the outer layer. × 360 diameters.

Fig. 18. Experiment DF_{17}. Section 30 micro mm. caudal to above, showing similar position of optic nerve. × 360 diameters.

Fig. 19. Experiment DF_{17}. Section 40 micro mm. caudal to above, showing optic nerve running from outer layer into medulla caudal to otic vesicle. The optic nerve-bundle can only be followed a few sections in the medulla. × 360 diameters.

Fig. 20. Experiment DF_{104}. Embryo rana palustris killed 19 days after transplantation of the optic vesicle into the region between the eye and the otic vesicle. Section through middle of transplanted eye and side of brain. The transplanted eye shows invagination and differentiation of the layers of the retina, the cup cavity and pupil are much smaller than normal, and there is no trace of a lens. The ganglionic layer bordering the cavity has in places only a few scattered cells as most of the cells form a heap projecting into the cavity and from this heap of cells a nerve arises which passes across the cavity through the narrow pupil out into the mesenchyme to the subectodermal pigment band. There is also a small optic nerve passing through the retina into the outer layer, the figure shows its position here as projected from the two neighboring sections. It can be traced for a short distance only in the outer layer. × 90 diameters.

Fig. 21. Experiment DF_{83}. Embryo rana palustris killed 18 days after transplantation of the optic vesicle into the region between the otic capsule and medulla. Section through transplanted eye, otic capsule, and medulla. The eye, owing to irregular invagination, has only a very narrow pupil and small, irregular cup cavity. Most of the optic nerve-fibers pass from the irregular ganglionic layer through the narrow pupil into the mesenchyme ventral to the otic capsule, where it splits into two divisions, the ventral one of these runs a short distance and ends abruptly in the mesenchyme, the other runs to the cartilage ventral to the otic vesicle and can be traced for a short distance along it. The peripheral portion of the nerve, as shown in the figure, is projected into the section from the neighboring sections. Another portion of the optic nerve, smaller than the first one, runs through the retina and seems to end abruptly at the pigment layer. This portion of the nerve has also been projected into the figure from a neighboring section. × 90 diameters.


14
15
19
20
17
18
21
16

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE EYE IN AMPHIBIA.

III. ON THE ORIGIN AND DIFFERENTIATION OF THE LENS.

BY

WARREN HARMON LEWIS.

Associate Professor of Anatomy, Johns Hopkins University.

WITH 83 FIGURES.

INTRODUCTION.

Since the publication of my paper on the origin of the lens in rana palustris,[1] I have made many new experiments on lens-formation with regenerating and transplanted eyes, not only in rana palustris but in rana sylvatica and amblystoma punctatum. My pupil, Mr. Le Cron, has also made experiments in the same field on amblystoma.[2] These new experiments confirm the conclusions given in my previous paper for rana palustris and throw additional light on the origin and early development of the lens. They leave no doubt, I believe, but that a lens, arising from the ectoderm in the amphibian embryo, is dependent for its origin on the contact influence of the optic vesicle on the ectoderm, in other words, the lens is not a self-originating structure. These experiments indicate that actual contact between optic vesicle and ectoderm is essential, and that the optic vesicle has not the power of acting at a distance to stimulate lens-formation. The size of the lens-plate, the lens-bud, the lens vesicle, and the early stages of the lens are shown to be dependent in part upon the actual area of this contact between optic vesicle and ectoderm. These experiments indicates also that not only is the lens dependent on the influence of the optic vesicle for its initial origin, but that its subsequent growth and differentiation is dependent on the continued influence, probably contact influence, for a time at least, of the optic vesicle. Le Cron's experiments were directed more especially towards this point and show that in amblystoma the influence of the optic vesicle must be exerted for a considerable period

[1] Am. Jour. of Anat., Vol. III, 1904.
[2] Am. Jour. of Anat., Vol. VI, 1907.

of time in order that a perfect lens may form. My experiments throw some light on the nature of the earliest influence of the optic vesicle on the ectoderm; lens-like structures of the ectoderm can be produced by mechanical injuries of the ectoderm, as with a needle or other instrument. These lens-like structures consist merely of a proliferation of cells of the inner layer of the ectoderm into small buds, small solid bodies or small vesicles, but they do not show signs of differentiation into lens fibers, etc. Their great similarity to some of the earlier stages of abortive lens-formation suggests the idea that the initial stimulus of the optic vesicle is such as to cause at first only an increase in the rate of cell division in the area of contact of the ectoderm, and it may be that the earliest stimulus of the optic vesicle is purely mechanical. It was shown in my previous paper that probably any portion of the inner layer of the ectoderm is capable of giving rise to a lens when properly stimulated. There is, then, no especial predetermined group of cells which must be stimulated, in order that a lens may arise. There cannot be then in the ovum or fertilized egg, substances, either protoplasmic or chromatic, or otherwise, which represent the lens in the sense in which Conklin has found certain definite kinds of protoplasm that differentiate into the central nervous system, the muscular system, etc. Such tissues or organs as the central nervous system, the muscular system, the ectoderm, etc., which are in a way represented by substances in the egg, possessing more or less power of self-differentiation, might be designated as fundamental or primary tissues and the others as the lens and cornea as secondary tissues. The latter would be dependent for their origin on reactions between the primary tissues during the course of development, or if the reaction takes place very early, between substances which represent them in the ovum. How large this group of secondary tissues is can only be determined by experimentation. That the cornea belongs to this class has been clearly shown by my experiments.[3]

The present paper is concerned for the most part with the effects on lens-formation after total or partial extirpation of the optic vesicle with total absence or varying degrees of regeneration of these eyes.

Anatomy of the Eye Region at the Operating Stage.

In embryos of rana palustris and rana sylvatica at about the time of, or shortly after, the closure and beginning fusion of the neural folds, the optic vesicle projects from the sides of the brain and produces a

[3] Jour. of Expt. Zoöl., Vol. II.

bulging of the ectoderm on the surface of the embryo (Figs. 1, 2, and 3). The cavity of the optic vesicle communicates with the brain cavity by a wide opening and there is very little indication of the formation of the optic stalk. The ganglionic mass of the fifth nerve lies in direct contact with the posterior surface of the optic vesicle and partially covers it. The walls of the optic vesicle are of about the same thickness as the ventral wall of the brain. There is at this time no indication of any differentiation between those cells which are destined to form the eye, or the optic stalk and the brain. That there are differences not brought out by the ordinary histological methods is evident from the results of my experiments. Among the embryos allowed to live about the same length of time after partial or total extirpation of the optic vesicle, there are regenerated eyes of all sizes, ranging from those of nearly normal size, to complete absence. In some, only the optic stalk has regenerated, and in others the brain wall may be defective from loss of tissue, which was cut away with the optic vesicle. Correlated with these differences in the regenerated eyes are differences in the transplanted eyes. In most of the embryos the optic vesicle, after having been cut away, was transplanted into the otic region of the same or another embryo, and among these transplanted eyes there are small ones and large ones, and some with bits of brain tissue attached. Such results are most readily explained if we assumed that those cells which go to form the eye are already determined and that the line of separation between brain and eye cells is sharp and can be indicated by a line, as *cd* (Fig. 3[a]). Cuts separating the eye from the brain lateral to *cd* would leave varying numbers of optic vesicle cells attached to the brain and so give rise to regenerated eyes of various sizes, cuts along the plane *cd* would leave no cells for regeneration, while cuts median to the plane *cd* would include, with the transplanted eye, various amounts of brain tissue. As all these conditions are found, in the embryos experimented upon, it is but natural to conclude that the eye cells are already predetermined, although on microscopical examination no line of demarcation can be seen between brain and eye cells at the time of closure of the neural folds. This question will be more fully discussed in another paper on the origin and differentiation of the optic vesicle.

The lateral surface of the optic vesicle at this stage is in direct contact with the inner layer of the ectoderm over a considerable area (see Fig. 3). There is very little mesenchyme at this side of the brain, but ventral to the brain in this region there is a considerable layer of mesenchyme. There are no indications of any changes in the ectoderm

leading to lens-formation, nor do these changes appear for some little time. That is, no indications are to be seen by the ordinary histological and embryological methods. The cells of the inner layer of the ectoderm are apparently all alike in this region. That there may be invisible differences in the cytoplasm or in the nuclei, or differences which have not as yet been recognized, is, of course, possible, but the following experiments would seem to indicate otherwise.

Thus the optic vesicle will continue its differentiation independently of any especial environment, as when transplanted into various regions of the embryo. Not, so, however, does the normal lens-forming ectoderm behave when its special environment is altered by removal of the optic vesicle, for without the influence of the optic vesicle no traces of the lens appear.

If the lens-forming ectodermal cells are different—the difference only appearing as development progresses—we should expect this to appear independently of their special environment (the presence of the optic vesicle) unless this environment is a necessary factor in bringing about their progressive differentiation, and as such environment is necessary the lens cannot be considered as a self-originating structure. It is only by the experimental method that we can so alter the normal environment as to afford a means of solving such questions. The elimination of the possible influence of the optic vesicle is naturally the first factor to be considered.

Method of Operation.

The embryos were operated upon under the binocular microscope. They were placed in small glass dishes either in ordinary tap water or in a 0.2 per cent salt solution. The latter solution does not offer any especial advantages, however. The embryo was held with a small pair of forceps and a semi-circular incision made, through the ectoderm caudal in the bulge produced by the optic vesicle, with a fine pair of scissors or a sharp needle. The skin flap thus formed was turned forward and left attached anterior to the eye. The optic vesicle and surrounding structures were thus exposed without injury either to them or the overlying ectoderm. In Fig. 2 a much larger skin flap than was used in the operation is shown turned forward from over the optic vesicle and ganglionic masses. At a later stage the operation of turning forward the skin flap becomes quite difficult, owing to adhesion which takes place between optic vesicle and ectoderm preceding lens-formation.

With the point of a fine needle or a small pair of scissors the optic vesicle was cut off close to the brain, thus leaving a large opening into the ventricle.

After removal of the optic vesicle the skin flap was returned into its original position and held in place for a few minutes either by the pressure of short pieces of silver wire, or better, by turning the embryo over, with the skin flap against the bottom of the dish, the weight of the embryo above being sufficient to hold the flap in place. There is often more or less contraction of the skin flap, so that it does not always cover the entire denuded ana. Healing takes place quite rapidly and in an hour or two the process is usually complete. The embryos were kept in small glass dishes and the water changed every day or two. The embryos were killed in Zenker's fluid at periods varying from 2 to 20 days after the operation, embedded in paraffine, cut into serial sections 5 to 10 micro mm. in thickness and stained in hæmatoxylin and congo red.

As great care was taken not to injure the skin flap, and especially that portion of the inner layer of the ectoderm which would, under normal conditions, have given rise to the lens, it seemed probable that the lens would arise unless it were in some way dependent for its origin directly or indirectly on the presence of the optic vesicle. It is easily shown that the mere turning forward of the skin flap from over the optic vesicle and then replacing it does not interfere with lens-formation, and even if a portion of the optic vesicle is cut away the remainder will regenerate an eye, and a lens will form from the ectoderm, provided, however, that the regenerated eye comes into contact with the ectoderm (see Figs. 67, 69, 71, 72, 73, 74, and 76).

Results from Experiments.

Absence of Lens-formation After Total Extirpation of the Optic Vesicle.

In 50 of the embryos of rana sylvatica thus experimented upon and killed at from 3 to 16 days after complete extirpation of the optic vesicle, there was no regeneration of the eye and no indication of a lens or of lens-formation in the normal lens region from which the eye was taken. Fig. 4, from an embryo killed 3 days after complete extirpation of the eye, shows complete absence of the eye and lens. Remnants of the optic stalk are imbedded in the ventral side of the brain. The normal eye on the opposite side of the head shows a large optic cup and lens (Fig. 5). Fig. 6 is from a section through the eye region of

another embryo killed 5 days after complete extirpation of the optic vesicle. A portion of the optic stalk has regenerated, but there is no trace of a lens in this region.

This resulting absence of lens-formation after complete extirpation of the optic vesicle in rana sylvatica is in entire accord with my previous work on rana palustris.[4] Some new experiments on rana palustris give me now 21 examples of complete extirpation of the optic vesicle by the above operation, in which there was failure of lens-formation, associated with complete absence of the eye.

Similar operations on amblystoma punctatum by Le Cron[5] likewise demonstrate the lack of lens-formation after complete extirpation of the optic vesicle at this early stage.

Spemann's experiments[6] on rana fusca, made on embryos younger than those used by me, also show that if the eye spot is killed on the open medullar plate, the lens fails to appear. In none of my experiments are there to be found lenses or lens-like structures in the normal lens region when the optic vesicle fails to regenerate.

As the lens often does arise when the eye regenerates its absence is not due to the operation itself but to the lack of influence of the extirpated optic vesicle. These experiments indicate very clearly that the lens is not a self-originating structure.

Absence of Lens-formation After Partial Extirpation of the Optic Vesicle.

In 51 of the embryos of rana sylvatica there was more or less regeneration of the eye without any indications, however, of lenses or beginning lens-formation. Figs. 7, 8, 9, and 10 are from sections through such regenerating eyes of embryos killed from 3 to 5 days after the operation. They are among the larger of the regenerating eyes without lenses, but are much smaller, however, than normal eyes of the same age (see Figs. 8, 75, and 77). These regenerating eyes are separated from the ectoderm by mesenchyme and were probably never in contact or not in contact with the ectoderm for a sufficient length of time to stimulate lens-formation. The majority of regenerating eyes without lenses are separated from the ectoderm by mesenchyme; a few exceptional ones, however, were found to be in contact with the ectoderm (Figs. 11, 12, and 13). The embryos from which these figures were taken

[4] See figure 6, p. 510, and figure 9, p. 512, Am. Jour. of Anat., Vol. III, 1904.

[5] Am. Jour. of Anat., Vol. VI, 1907.

[6] Verhandl. der Anat. Gesell., 1901.

were killed 3 days after the operation, and on the normal side in each is a well-formed lens (Fig. 59). The regenerating eyes in these embryos are in contact with the ectoderm by the outer layer, which does not seem to possess the power of stimulating lens-formation.

In 34 embryos of rana palustris killed at varying ages after partial extirpation of the optic vesicle there are regenerating eyes of various sizes without lenses or traces of lens-formation.

Regenerating eyes in contact with the ectoderm for a sufficient length of time can stimulate lens-formation, but in the above experiments the absence of lens-formation is to be explained through want of contact between eye and ectoderm, or to the contact not having been of sufficient duration; or to the contact having been over too small an area, or to contact by the outer layer of the optic vesicle, which does not seem to possess the power of stimulating lens-formation. The adhesion which ordinarily takes place between optic vesicle and ectoderm before lens-formation is probably an important factor, and if this is interfered with even though contact may exist, it is possible that a lens would not arise.

These experiments indicate that the lens is not self-originating, and that the regenerating eye cannot stimulate lens-formation when separated from the ectoderm by mesenchyme.

Lens-formation Associated with Regenerating Eyes.

In 25 embryos of rana sylvatica killed from 3 to 16 days after partial extirpation of the optic vesicle there are regenerating eyes of various sizes associated with lenses or lens-like structures of various stages[7] and sizes (Figs. 17, 27, 30, 34, 35, 36, 37, 64, and 65).

Small and imperfect lens structures are often associated with some of these smaller regenerated eyes. The larger regenerated eyes, however, give rise to normal lenses, indicating thereby that the operation itself, unless a considerable portion of the optic vesicle is cut away, does not interfere with lens-formation from the skin flap. These large re-

[7] It has seemed convenient to divide the development of the lens into several stages: (1) the lens-plate, or the thickening of the inner layer of the ectoderm; (2) the lens-bud (Fig. 67), projection of this thickened area until its separation from the ectoderm; (3) the lens-vesicle (Figs. 43, 59, 66, 68, and 69), the vesicle-like structure from the time of its separation from the ectoderm until the differentiation of the anterior epithelial layer and lens-fibers; and (4) the lens (proper), the earlier stages of which are seen in Figs. 61 and 70, and the later stages in Figs. 38, 74, 75, 76, 77, and 78.

generated eyes with lenses in rana sylvatica are very much the same as in rana palustris (see Figs. 67, 69, 73, 74, and 76).

The sizes of the regenerating eyes during these early stages seem to be dependent much more upon the amount of eye tissue left attached to the brain, during the operation, than upon the length of time the embryo is allowed to live.

In 76 embryos of rana palustris lenses or abortive lenses of various sizes and stages are associated with regenerating eyes. In some of the smaller regenerating eyes the lens-bud or vesicle is very small and does not show much differentiation (see Figs. 18, 19, 20, 21, 23, 24, 25, 26, 31, 32, 33, 40, 41, 60, 61, 62, 63, 64, and 65. In the larger regenerated eyes the lens-buds, or vesicles, or lenses approach more the normal, as in Figs. 67, 69, 71, 72, 73, 74, and 76. These experiments indicate, as in rana sylvatica, that the failure of lens-formation, when the eye fails to regenerate or only regenerates a little, is not due to the mere reflecting of the skin flap and replacing the latter, but to the lack of the proper stimulus to the ectoderm. There is every reason to believe that in these experiments the regenerating eyes were in contact with the ectoderm and then stimulated lenses to form, their sizes depending upon the area of contact between optic vesicle and ectoderm and upon the duration of this contact. And the fact that the eye, after the lens has been stimulated to arise, is separated from the ectoderm by mesenchyme is no indication that they were not at one time in contact. I see no other way of explaining why some regenerated eyes are without lenses and others have them, except in this manner, as the location of two eyes may be almost exactly the same at the time of killing the embryo, yet one may have a lens and the other not.

Abortive Lenses with Regenerating Eyes.

In both rana palustris and sylvatica there are a number of very curious small lens-buds associated with the small regenerating eyes (Figs. 18, 19, 20, 21, 23, 24, 25, 26, 27, and 30). Such abortive lens-buds are merely solid outgrowths of the inner layer of the ectoderm, and are very much smaller than the normal lenses; they show no especial indication of differentiation into lens-like structures, yet they were undoubtedly caused by the small regenerating eyes. Figs. 18, 20, 23, 36, and 37 show only small and imperfect areas of contact between optic vesicle and ectoderm, and to this is due, in part, the small size and imperfect development of the lens-buds. The ingrowth of mesen-

chyme between the small lens-buds and eye, as seen in Figs. 25, 27, 30, 34, 35, 57, and 58 is an additional factor in causing these abortive lenses. Some of these lens-buds would probably have remained attached to the ectoderm for many days, while others, such as shown in Figs. 21, 26, 27, 31, 32, 33, 36, and 37 might ultimately have separated, to form small solid spherical masses, as shown in Figs. 40, 41, and 42. Figs. 14, 15, and 17 show how the mesenchyme may grow in between the early lens-plate and the optic vesicle. These lens-plates would probably have soon ceased to develop and small lens-buds have formed from them.

Somewhat larger regenerating eyes give rise to larger lens-buds and vesicles, as in Figs. 60, 61, 62, 63, 64, 65, and 67. When such lens-structures retain more normal relations with the optic cup they will develop into small but fairly normal lenses, as in Figs. 71 and 72.

The larger regenerating eyes give rise to more normal lenses, as in Figs. 69, 73, 74, and 76.

These experiments indicate that the lens is neither self-originating nor self-differentiating, but is dependent for its origin, its size, its differentiation, and its growth on the influence of the eye.

Abortive Lens-formation with Degenerating Eyes.

Among these experiments there are four rather fortunate examples of degeneration and partial disintegration of the brain and the eye on the unoperated left side of the head. Three of these embryos were operated upon for extirpation of the right eye in succession and were killed 4 days after the operation. The fourth (DL_1) was operated upon at an earlier time. In each the supposedly normal eye is much smaller than a normal one of the same age, and shows only shallow invagination, no differentiation of the layers of the retina except the outer pigment layer. Compare Figs. 79, 80, 81, and 82 with a normal eye (Fig. 77), from an embryo killed 4 days after the operating stage and of about the same age. The important point in connection with these degenerating eyes is in the size and differentiation of the lenses. Instead of a large lens, as in Fig. 77, with long lens-fibers and a well developed epithelial layer we have only small lens vesicles. In experiment DL_{91} there are two such lens vesicles (Figs. 81 and 82) associated with the same eye. The lens vesicles, although small and retarded in differentiation, show no signs of degeneration. They are very similar to some of the small lens vesicles associated with small regenerated eyes (compare with Figs. 60, 61, 62, 63, 64, and 65). What I imagine has taken

place is: that something connected with the operation started progressive degenerative changes in the optic vesicle, but before they had become much advanced a small lens-plate and lens-bud were stimulated. Owing, however, to the increase in the degeneration the eye lost for the most part its usual influence on the developing lens, consequently the great retardation and apparent stoppage of the growth and differentiation of the later in the vesicle stage. These eyes seem to be rapidly disappearing and it is possible if one of the embryos had been killed a few days later the eye would have completely degenerated and disappeared, leaving a lens vesicle of unknown origin, and thus might have been mistaken for a self-differentiating and self-originating structure.

In another experiment (t_{12}) in which tissue from another embryo was transplanted into the otic region without disturbing in any way mechanically the optic region both eyes and the brain were found to be degenerating, and associated with each eye is a small abortive lens vesicle similar to the ones shown in Figs. 77, 80, 81, and 82. The embryo was killed 6 days after the operation. The embryo, at the time of the operation, was of the same age as in the other experiments, consequently there is even more difference between the size and degree of differentiation of these two abortive lenses and normal ones of the same age than between those shown in Figs. 79, or 80, and 77.

Lens-like Structures Due to Mechanical Injury of the Ectoderm.

In many of the embryos experimented upon, especially in rana sylvatica, but also in rana palustris and amblystoma, ectodermal buds project into the mesenchyme from places on the ectoderm liable to have been injured during the operation (Figs. 45, 46, 47, 48, 49, and 50). Such buds are found anterior to the regenerating eye, or posterior to it, more often, however, in the region of the otic capsule. The injury bud in Fig. 48 is very similar to the lens-buds of Figs. 19 and 58, and to the ectodermal bud in Fig. 44. The latter was probably formed at the place where the normal lens was pinched off from the ectoderm.

In making the pockets beneath the ectoderm for the transplanted eyes, the overlying ectoderm was often injured with the needle. Also in transplanting the eyes small pieces of ectoderm, either from such wounds or from the edge of the incision, were often pushed into the mesenchyme. The smaller ectodermal bodies take on a solid spherical form, as in Fig. 51 and resemble very much the small lens-like bodies of Figs. 40, 41, and 42, caused by the influence of small optic vesicles

on the ectoderm. The larger ectodermal bodies form vesicle-like structures, as in Figs. 52, 53, 54, 55, and 56. Some of these bear a very close resemblance to small lens vesicles, associated with small regenerating eyes.

This marked similarity between some of the early abnormal lens-buds or vesicles and those caused by injury suggest, of course, the idea that the initial stimulus of the optic vesicle on the ectoderm is one which merely causes those cells of the inner layer to multiply more rapidly than normal. The cells of the inner layer of the ectoderm seem to have the power of responding to the stimulus of the optic vesicle, or to the mechanical stimulus of the point of a needle, by increased rate of cell division. There is also a tendency for these groups of cells to hold together in the form of buds, or spherical masses, the latter being hollow when large, forming vesicles. In many of the embryos, especially in rana sylvatica, these injury processes are often very long and large and seem to result partly from accidental transplanting an attached piece of ectoderm into the mesenchyme; they may extend for long distances into the mesenchyme, and when caudal to the otic capsule often unite with the pharyngeal epithelium. When they occur in the region of a regenerated or transplanted eye, the entire process, or only a portion of the process, may undergo transformation into a lens, provided it comes into contact with the eye, but not otherwise. These will be considered later in connection with lens-formation from transplanted eyes.

Discussion of Results from the Experiments.

Is the Lens Self-originating?

These experiments point very clearly to the lens not being a self-originating structure. Its entire absence after total extirpation of the optic vesicle without regeneration of the eye, and its absence in many of the embryos with small regenerating eyes are very conclusive evidence in favor of this view. The small abortive lenses with small, irregular, regenerating eyes and the evident dependence of the lens for its growth and differentiation on the continued influence of the optic cup, and Le Cron's experiments on amblystoma showing that the lens is dependent on the continued influence of the optic cup for its growth and differentiation even after it has separated from the ectoderm, also support this view.

These results are not in accord with the conclusions of King.[8] On

[8] Experimental Studies on the Eye of the Frog. Arch. f. Entwirklungsmerk. d. Organ., XIX, 1905.

page 97 we find the following statement relating to her results: "These results apparently furnish evidence in favor of the view that in a definite region of the ectoderm the cells are destined to form a lens, and therefore they will produce such a structure even if an optic cup is lacking;" and on page 99: "It appears that the power of self-differentiation must be granted lens-forming cells of the ectoderm in the embryo of rana palustris." King bases this view on the appearance of "lens-like structures" attached to the inner layer of the ectoderm directly opposite the place where the lens is forming for the normal eye. On the side of the head where these "lens-like structures" are forming there is absolutely no trace of an optic cup, the latter having been killed by puncturing with a hot needle. The very nature of King's operation makes us at once suspicious of these "lens-like structures." The puncturing the side of the head with a hot needle is a very uncertain and crude mode of operation and as King says (p. 93): "Presumably the operation destroyed that portion of the ectoderm that would normally produce a lens." In these operations, which were done on embryos somewhat younger than those used by me, it would seem to me also quite impossible to destroy the optic vesicle completely by puncturing the side of the brain with a hot needle without destroying completely those ectodermal cells which King supposed are predestined to differentiate into a lens. If these cells are killed then by operation, King's whole argument falls to the ground. Operating on these embryos with a hot needle to such an extent as to completely or almost completely kill the optic vesicle and part of the brain is a very severe mode of procedure, and the after effects of such operations as King's must be quite extensive, as about one-half of the embryos died during the first day. It is possible that King may not have destroyed, in some instances, at the time of the operation, completely, the optic vesicle, and that the remnant left may have started the lens-like bud before the complete degeneration, disintegration, and disappearance of the eye resulted from the after effects of the operation. I have already called attention to abortive lens-formation, associated with degenerating eyes, and with a complete degeneration of the eye, such a condition as King found, might readily occur, especially when we consider what Le Cron found in amblystoma, namely, that the early stages of lens-development and differentiation are dependent on the continued influence of the optic vesicle or optic cup, for if the eye is removed without injury to the lens-plate or lens-bud, these structures soon cease to grow and differentiate; the earlier the eye is removed the less power the lens rudiment has of progressive

self-differentiation.[9] Again among my experiments on rana palustris and rana sylvatica there are numerous instances in which the lens-bud has apparently ceased to develop, owing, I believe, in part, at least, to a shifting away from the bud of the small regenerated eye, or irregular transplanted eye, and to an ingrowth of more or less mesenchyme between the two; in others irregular changes in the form of the eye which shifts the contact of the lens from the retinal to the outer layer leads to similar abortion in lens growth (see Figs. 58, 57, 18, 20, 25, 27, 30, 34, and 40.

Another possible explanation of King's "lens-like structures" is that they are injury buds due to injury of the ectoderm during the operation, and are so, perhaps, similar in origin to some of those I have already called attention to in a preceding section.

My experiments on the extirpation, partial or entire, of the optic vesicle, were performed on embryos a few hours older than those used by King. The possibility or probability that the lens-forming ectodermal cells possess at the stage King used any unusual powers of self-differentiation into a lens is scarcely worth serious consideration, as one would naturally expect this power of self-origination to show itself at the stage I used more readily than at the stage King used. Spemann's experiments were on embryos younger than those used by King, yet he had no indications of self-origination of the lens. In view of these various facts of abortive lens-formation it seems to me much better to explain King's lens-like structures in one of these ways rather than concluding that the lens is self-originating.

Can the Optic Vesicle Stimulate Lens-formation from a Distance or is Direct Contact of the Optic Vesicle with the Ectoderm Necessary?

We have noted that in 85 embryos, 51 in rana sylvatica and 34 in rana palustris, there were regenerating eyes of various sizes without lens-formation. Such eyes are usually separated by mesenchyme from the ectoderm, yet eyes may come into contact with the ectoderm by the outer layer without stimulating lens-formation. The regenerating eyes without lenses are usually smaller than those with lenses. Owing to the small size the mesenchyme is much more likely to grow in between the eye and ectoderm and then to prevent contact between the two. In

[9] Proc. Ass. of Am. Anatomists, December, 1906. Jour. of Anat., Vol. V, p. XI; also Am. Jour. of Anat., Vol. VI, No. 2.

some embryos an irregularity in the skin flap may have prevented its contact with the stump of the optic vesicle.

From a study of the abortive lenses and lenses of various sizes with regenerating eyes it becomes apparent that not only is contact between eye and ectoderm necessary for the initial origin of the lens, but that the size of the lens-plate and lens-bud is dependent upon the area of contact, also the development of the lens is dependent on the continued influence of the eye. The fact that a regenerating eye is often separated from the ectoderm by mesenchyme is no indication that it was never in contact with it. Mesenchyme always grows in between the normal eye and ectoderm (Figs. 77 and 78), yet one would not hesitate to state that at the time of lens-formation the normal eye was in contact with the ectoderm. The operations do not interfere very much with the growth of mesenchyme in the region of the regenerating eye, and as the regenerating eyes are smaller than normal and are attached to the brain, we should expect to find them more often separated from the ectoderm by mesenchyme than the normal ones, and also at a greater distance from the ectoderm than in normal eyes. This ingrowth of mesenchyme often interferes with lens-formation, if the ingrowth takes place before the lens has started the latter will fail to appear, but if the ingrowth of mesenchyme occurs after the lens has begun to form, various degrees of abortive lens-formation occurs, these depending, in part, on the stage of development of the lens-bud at the time of the ingrowth of the mesenchyme, separating the lens from the eye. In some instances the lens-bud may be pulled out into a long process, owing to the adhesion between eye and lens-bud. As the eye is attached to the brain the pressure of the growing mesenchyme would tend to force the ectoderm away from it and thus either stretch out the lens-bud or separate the eye from the lens-bud or the lens-bud from the ectoderm. Figs. 31 and 33 are from experiments where I believe the optic vesicle was originally in contact with the ectoderm, stimulated a small lens-plate and small lens-bud, but with the growth of mesenchyme the small eye has been pushed some distance from the ectoderm, and owing to the adhesion between the lens-bud and eye the former was pulled out into an elongated form. Following this has come more or less separation of the lens-bud and optic-cup, and owing to the original small size of the lens-bud and its later separation or partial separation from the optic-cup retardation in development has occurred. The embryos (DF_{43} and DF_{44}) from which Figs. 31 and 33 are taken were killed 4 days after the operation and are in contrast to the conditions found in another embryo

(DL_{61}, Fig. 71) which was also killed 4 days after the operation. As is seen in Fig. 71, the small regenerating eye and small lens are separated from the ectoderm by a considerable layer of mesenchyme. Here there was probably a larger area of contact between the regenerating eye and ectoderm than in embryos DF_{43} or DF_{44}, and so a larger lens-plate and lens-bud; there was also probably greater adhesion between optic vesicle and lens-bud, so that when the mesenchyme expanded the side of the head the lens remained with the eye. In Fig. 72 a similar condition is shown, where the area of contact between the small regenerating eye and ectoderm is very small and only a few cells enter into the lens-bud; the mesenchyme as it grows in between the eye and ectoderm tends more to separate the lens-bud from the eye than from the ectoderm, and such conditions as seem in Figs. 27, 30, 34, 57, and 58 occur. Some variations occur, of course, and the lens-bud may form a small vesicle or solid body which is separated from both ectoderm and eye, as in Fig. 40.

King has pictured a somewhat similar condition in rana palustris, where the lens-bud is separated by mesenchyme from the optic-cup [10] (Figs. 5 and 6). She, however, explains this condition by asserting that the "lens can be formed from the ectoderm when the optic-cup is some distance beneath the surface of the body," and that "contact between the optic-cup and ectoderm is not necessarily the stimulus that tends to the development of the lens." King's idea that the regenerating eye (Fig. 6) was never in contact with the ectoderm (p. 95) is based on a misconception of the conditions at the time of an immediately after the operation. There is, of course, at this early stage very little mesenchyme in the eye region (see Fig. 3), and the ectoderm lies rather close to the side of the brain and the developing eye, and I believe that in the early stages of the regenerating eyes in King's experiments they must often have come into contact with the ectoderm and in some embryos have stimulated lens-formation. The growth of the mesenchyme here, as in my experiments, would tend either to separate the lens-bud and optic-cup or to elongate the lens-buds, as in King's (Figs. 5 and 6).

It would seem better to explain King's lens-like structures to have been formed in some such manner rather than to assume that the optic vesicle can act at a distance, especially when we consider the fact that these regenerated eyes of various sizes may remain at varying depths beneath the normal lens-forming ectoderm for from 3 to 18 days without any signs of lens-formation appearing, and so indicate quite clearly

[10] Roux's Archiv, Bd. 19, Taf. VI.

that action at a distance is a process not very likely to occur. The larger the regenerated eyes the greater the number that show lenses. This is what one would expect if contact were necessary, for the larger the regenerated eye the greater its chance for prolonged contact with the ectoderm.

We are practically forced to conclude that actual contact between optic vesicle and ectoderm is necessary for lens-formation, as this seems to be the common factor lacking in the above 85 examples of regeneration of the eye without lens-formation.

The lens is then dependent on direct contact of the retinal portion of the optic-cup or vesicle on the inner layer of the ectoderm for its origin. Abnormal relation between the early lens-plate or lense-bud and eye is accompanied by more or less abnormal or abortive lens-formation. The size of the lens, as will be shown further on, and even the differentiation of the lens, are dependent on the continuance of the normal relations between the optic-cup and developing lens.

The Lens is Not Self-differentiating.

An optic vesicle transplanted into the region of the otic vesicle, for example, will continue its growth and differentiation independently of any especial environment. Invagination, differentiation of the various layers of the retina, and the formation of the optic nerve takes place as readily as when the eye has its normal position and attachment to the brain. It is a remarkable self-differentiating organ. The behavior of the lens is in marked contrast to eye. Le Cron has shown that in amblystoma removal of the optic-cup without injury to the developing lens is followed by abortive lens-formation. The earlier the stage at which the eye is removed the less power of growth and differentiation the lens rudiment possesses. Even when the optic cup is removed after the lens vesicle has separated from the ectoderm its progressive growth and differentiation soon cease and ultimately degenerative changes occur in the lens-fibers.

The numerous instances of abortive lens-formation in rana are conclusive evidence that here too the lens is not a self-differentiating structure, but is dependent, not only on the presence of the optic-cup, but the maintenence of more or less normal relations between the two for a considerable period of time. Disturbance of such relationship by irregular invagination or lack of invagination of the optic vesicle and by ingrowth of mesenchyme results, as in complete extirpation of the optic cup, in abortive lens-formation.

The Size of the Early Stages of the Lens Dependent in Part Upon the Area of Contact Between the Eye and Ectoderm.

Associated with the smallest of the regenerating eyes are the very small lens-plates, as in Figs. 15 and 17, or small lens-buds, as in Figs. 18, 19, 20, 21, 23, 24, etc. Here the area of contact between optic vesicle and ectoderm must have been much smaller than in the normal. With the somewhat larger regenerating eyes we find somewhat larger lens-buds and vesicles, as in Figs. 60, 61, 62, 63, 64, and 65. Still larger eyes show larger lens-buds or vesicles, as in Figs. 67, 69, 71, 72, and 73. Regenerating eyes which approach the normal eye in size have lenses, nearly normal in size, as in Figs. 74 and 76. These differences in the sizes of the lenses, as well as in the sizes of the regenerating eyes are not due to differences in ages of the embryos, for if corresponding stages in the differentiation of the lens are compared it is found that the actual sizes of the lens-plates in small regenerating eyes is much smaller than a normal lens-plate, that the lens-buds vary in size somewhat according to the size of the regenerating eyes—the lens vesicles also and the early stages of the lens proper likewise. In some instances the small sizes are to be accounted for by loss of the continued influence of the optic vesicle, as when the latter is separated from the lens structure by mesenchyme. These differences in sizes are due in great part to the fact that the number of cells influenced to take part in the formation of the lens-plate and lens-bud is probably directly dependent upon the area of contact between the ectoderm and the retinal portion of the eye. In general the area of contact will vary with the size of the eye. Owing, however, to irregularities in the position and shape of the regenerating eyes it might often happen that the area of contact would be either larger or smaller than the usual relation of this area to the size of the optic vesicle. Hence the size of the early lens is not always in proportion to the size of the eye. The lens, for example, in Fig. 73 is larger than in 71 or 72, but the optic-cup is smaller. The adhesion which normally takes place between optic vesicle and ectoderm preceding lens-formation and accompanying it is probably an important factor and it is possible that without such adhesion lens-formation from the ectoderm will not follow. The area of adhesion may not, perhaps, always be as large as the area of contact. As, for example, the contact between the outer layer of the eye and ectoderm is not followed by lens-formation. Whether the outer layer will adhere to the ectoderm or not I am unable to say, but presume not, as this would be the most ready

explanation of contact without lens-formation, provided lens-formation is dependent upon adhesion. The size of the lens-plate and lens-bud, that is the number of cells of the inner layer of the ectoderm which take part in their formation, is dependent then, upon the area of contact of the retinal portion of the eye and probably upon the area of adhesion between the retinal portion of the eye and the ectoderm.

The size of the lens-bud and lens vesicle is dependent also upon the increase in the number of cells, and this is dependent upon the continued influence of the optic-cup. For how long a period contact is necessary or for how long a time the lens must maintain its normal relations with the optic-cup in order that it may develop and grow independently I am unable to answer at present. Whether a very small lens-bud would ever form a normal sized lens, even if its relations with the small optic-cup were maintained as in a normal eye for a long period, I am also unable at present to determine from my specimens.

Still other factors may play a rôle in regulating the size of these lenses as retarded stimulation of the ectoderm by the regenerating eye. It is possible, of course, that as the embryo gets older the ectoderm responds less and less readily to the stimulation of the optic vesicle, or that older optic vesicles stimulate less readily the ectoderm to form lenses. It is not possible, however, to determine this from my experiments.

The Nature of the Initial Stimulus of the Optic Vesicle Leading to Lens-formation.

Under normal conditions that portion of the optic vesicle which forms the retina first comes into contact with the inner layer of the ectoderm, the two soon become adherent and they can only be separated with difficulty. Then follows a thickening of this inner layer, which is greatest nearer the center of this area of contact. Accompanying this thickening and probably in part, at least, the cause of it, is an increase in the number of cells in this area. In order at first to accommodate the increase in the number of cells they, through mutual lateral pressure on each other, are compressed in the axis parallel to the ectoderm and elongated in the perpendicular axis. This gives the formation of the lens-plate. Accompanying this thickening there takes place the invagination of the optic vesicle. The invagination of the optic vesicle into the form of the optic-cup is an active process on the part of the optic vesicle, as I have already pointed out.[11] As the cells of the lens-plate

[11] Am. Jour. of Anat., Vol. III, Proc. of the Ass. of Am. Anat., p. XIII.

increase in number they project more and more into the optic-cup cavity and form the lens-bud. It is possible, owing to the adhesion of the lens-plate and lens-bud to the retinal layer of the actively invaginating optic-cup that the latter exerts a pull on the lens-plate and helps in the evagination of the lens-bud. This same pull may also be the stimulus which causes the cells to multiply as they are elongated. These are, of course, very difficult points to prove.

The first and most important early influence then which the optic vesicle exerts on the inner layer of the ectoderm is a stimulus causing the cells over the area of contact, and especially over the center of this area, to multiply faster than those in the region about. This is clearly indicated by several facts. There is at first no apparent alteration in the constitution of lens-plate or lens-bud cells. The cells of some of the small abortive lens-buds even several days after their formation are similar in staining reactions to the cells of the inner layer of the ectoderm. There is a difference in shape, but this is probably due to mechanical relations. Again the cells of the abortive lens-buds or vesicles are similar to the lens-buds or vesicles arising from mechanical injuries of the ectoderm. The fact also that these early lens-buds are not self-differentiating, points to the cells not being essentially different in structure from the cells of the inner layer.

That the prolonged influence of the optic-cup does alter the structure and chemical constitution of the lens-cells would seem self-evident.

Conclusions.

The lens will not arise from the normal lens-forming region of the ectoderm without the contact stimulus of the optic vesicle on the inner layer of the ectoderm. The lens is not a self-originating structure.

The lens will not develop, grow and differentiate, without the continued influence of the optic vesicle and optic-cup.

The lens is not a self-differentiating structure.

Probably only the retinal portion of the optic vesicle is capable of stimulating lens-formation from the ectoderm.

The size of the early lens-structure is due in part to the area of contact or adhesion between optic vesicle and ectoderm, and in part to the length of time the optic vesicle or optic-cup remains in contact by its retinal layer with the growing lens-structure.

The intitial stimulus of the optic vesicle on the skin is such as to cause increase in the rate of cell-division at the place of contact, and may be only mechanical.

The drawings were all made with the aid of a camera.

FIG. 1. Outline of rana sylvatica at operating stage. The neural folds are partly, or in some embryos, completely fused. × 12 diameters.

FIG. 2. Same embryo with large skin flap turned forward, exposing the brain with the optic vesicle, and the ganglionic masses of the cranial nerves. The ganglion of the fifth nerve partly covers the optic vesicle and was often partially removed. The skin flap is larger than the one usually used in the operations, the caudal edge of the operating flap being between the ganglia of the fifth and eighth nerves. × 12 diameters.

FIG. 3. Section through optic vesicle region of rana sylvatica at operating stage. The fusion of the neural folds in this region is not complete. The ectoderm as yet shows no signs of lens formation. × 90 diameters.

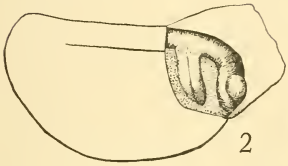
FIG. 3a. Outline of section through optic vesicles of rana sylvatica at operating stage. *cd*, position of cut for extirpation of the optic vesicle. × 22 diameters.

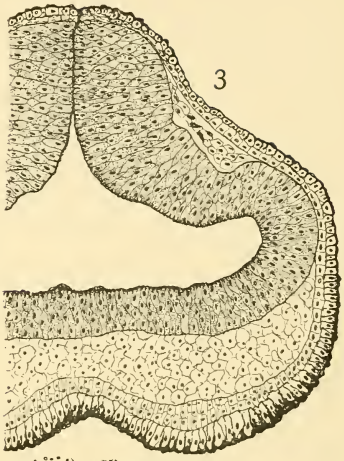
FIG. 4. Experiment DL_{11}. Embryo rana sylvatica killed 3 days after complete extirpation of the right optic vesicle. Transverse section through the right eye region. No traces of right eye or lens are to be found in the sections. × 90 diameters.

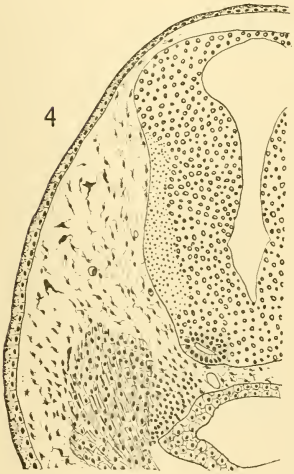
FIG. 5. Section through left normal eye and lens of above embryo (experiment DL_{11}). × 90 diameters.

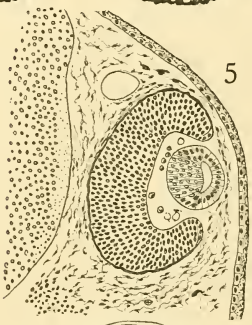
FIG. 6. Experiment DL_{37}. Embryo rana sylvatica killed 5 days after the operation. Transverse section through the center of the left eye, right eye entirely wanting, except for a bit of the optic stalk (*e*). There is no trace of lens formation on the right side of the head. × 45 diameters.

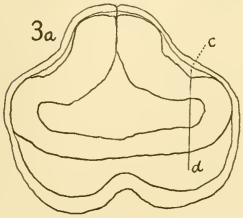

1


2


3


4


5


3a
c
d

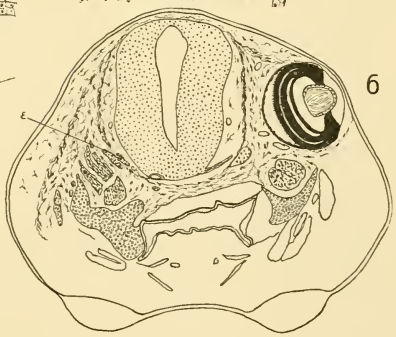

6
e

FIG. 7. Experiment DL_5. Embryo rana sylvatica killed 3 days after partial extirpation of the right optic vesicle. Transverse section through the small regenerated eye. There is no trace of lens formation. The normal left eye is like the one in figure 6. × 90 diameters.

FIG. 8. Experiment DL_{48}. Embryo rana sylvatica killed 5 days after partial extirpation of the right optic vesicle. Transverse section through small regenerated eye and through the normal left eye. No trace of a lens is to be found on the right side. × 45 diameters.

FIG. 9. Experiment DL_{22}. Embryo rana sylvatica killed 4 days after partial extirpation of the right optic vesicle. Transverse section through small regenerated eye. No traces of lens formation are to be found in the sections. Two sections caudal to this one the regenerated eye shows its attachment by a long optic stalk to the brain. The normal left eye is like the one in Fig. 77, experiment DF_{63}. × 90 diameters.

FIG. 10. Experiment DF_{64}. Embryo rana palustris killed 5 days after partial extirpation of the right optic vesicle. Transverse section through small regenerated right eye. No traces of lens formation are to be found in the sections. It is attached to the brain by a long optic stalk. The normal left eye is like the one in Fig. 75, experiment DF_{18}. × 90 diameters.

FIG. 11. Experiment dx_1. Embryo rana sylvatica killed 3 days after partial extirpation of the right eye. Transverse section through the regenerated right eye which is in contact by its outer layer with the ectoderm. (There is an artificial separation in the sections.) No traces of lens formation are to be found in the sections. The normal eye is like the one in Fig. 59, experiment DF_{68}. × 90 diameters.

FIG. 12. Experiment DL_2. Embryo rana sylvatica killed 3 days after partial extirpation of the right optic vesicle. Transverse section through the small regenerated right eye which is in contact with the ectoderm by its outer layer. No traces of lens formation are to be found in the sections. The normal left eye is like the one in Fig. 6, experiment DL_{11}. × 90 diameters.

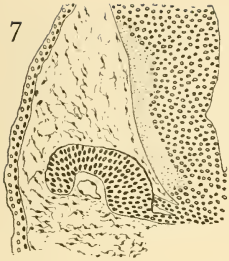
FIG. 13. Experiment DF_{73}. Embryo rana palustris killed 3 days after partial extirpation of the optic vesicle. Transverse section through small regenerated right eye which is in contact by its outer layer with the ectoderm. No traces of lens formation are to be found in the sections. The normal left eye is similar to that in Fig. 59, experiment DF_{68} or DF_1. × 90 diameters.

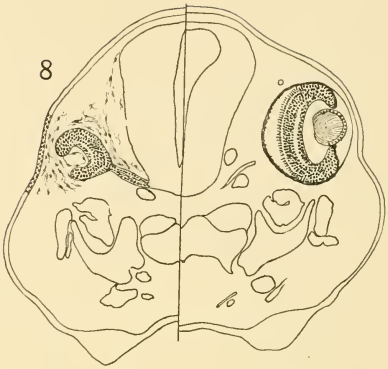
FIG. 14. Experiment DF_3. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Transverse section through the small regenerated right eye which is in contact by a portion of its retinal layer with the ectoderm. There is a slight thickening of the inner layer of the ectoderm opposite the eye but separated from it by a thin layer of mesenchyme. The normal left eye is similar to that in Fig. 59, experiment DF_{68}. × 90 diameters.

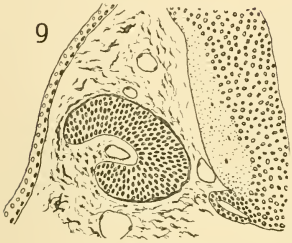
FIG. 15. Experiment DF_3. Section through the lens-plate more highly magnified than in Fig. 14. × 360 diameters.

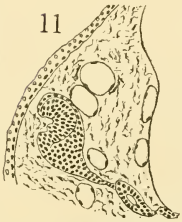
FIG. 16. Experiment DL_{40}. Embryo rana sylvatica killed 4 days after partial extirpation of the right optic vesicle. Transverse section through the caudal part of the small regenerated eye showing its connection with the brain. The normal left eye is similar to the one in Fig. 70, experiment DF_{10}. × 90 diameters.

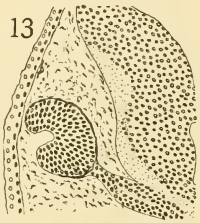
FIG. 17. Section through part of anterior end of the eye in experiment DL_{40} showing its contact with the ectoderm by the retinal layer and the formation at the place of contact of a small lens-plate. (The separation of the eye and ectoderm in the figure is an artefact.) × 180 diameters.

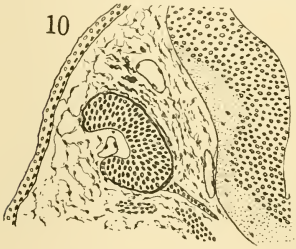

7

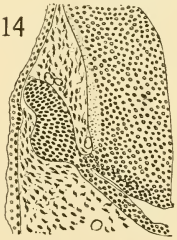

8


9

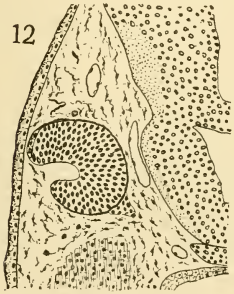

11

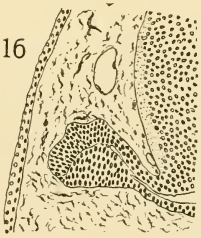

13


10


14


15


12


16


17

Fig. 18. Experiment DF_7. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through the small regenerated eye showing where outer layer and small corner of the retinal layer were in contact with the ectoderm. A small lens-bud is attached here to the inner layer of the ectoderm. The normal left eye is similar to the one in Fig. 77, experiment DF_{63}. × 90 diameters.

Fig. 19. Lens-bud in the above experiment DF_7, more highly magnified. × 360 diameters.

Fig. 20. Experiment DF_{72}. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Section through anterior portion of regenerated eye showing contact of edge of the cup with the ectoderm and the formation of a small lens-bud from the inner layer of the ectoderm. The normal right eye is similar to that in Fig. 42 or 68, experiment DF_{75} or DF_1. × 90 diameters.

Fig. 21. Lens-bud in above experiment, DF_{72}, more highly magnified. × 360 diameters.

Fig. 22. Section through caudal portion of same eye, experiment DF_{72}, show small cup separated from the ectoderm by mesenchyme. × 90 diameters.

Fig. 23. Experiment DF_{69}. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Section through caudal end of regenerated eye, it is separated from the brain by the anterior end of the transplanted eye and apparently pushed against the ectoderm where there is a small lens-bud attached to the inner layer of the ectoderm. The retinal portion of the regenerated eye is in contact with the lens-bud. The normal left eye is similar to the one in Fig. 42, experiment DF_{75}. × 90 diameters.

Fig. 24. Experiment DF_{69}. Lens-bud more highly magnified. × 360 diameters.

Fig. 25. Experiment DF_8. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through the small regenerated right eye. It is separated from the ectoderm by a thin layer of mesenchyme. There is a small lens bud attached in the inner layer of the ectoderm. The normal left eye is similar to that in Fig. 61, experiment DF_{48}. × 90 diameters.

Fig. 26. Experiment DF_8. Lens-bud more highly magnified. × 360 diameters.

Fig. 27. Experiment dx_3. Embryo rana sylvatica killed 3 days after partial extirpation of the right optic vesicle. Section through small regenerated right eye and small lens-bud, the latter is still attached to the ectoderm but is separated from the optic cup by mesenchyme. × 90 diameters.

Fig. 28. Experiment dx_3. Caudal end of regenerated eye in contact with the ectoderm by the outer layer. No signs of lens formation here. × 90 diameters.

Fig. 29. Experiment dx_3. Section through normal left eye. × 90 diameters.

Fig. 30. Experiment dx_4. Embryo rana sylvatica killed 3 days after partial extirpation of the right eye. The regenerated eye is about the size and shape of the one in Fig. 27. Its caudal end does not show the outer layer cells. Section through caudal end of the eye and lens-bud. A thin layer of mesenchyme separates them. The normal left eye is similar to the one in Fig. 29. × 360 diameters.

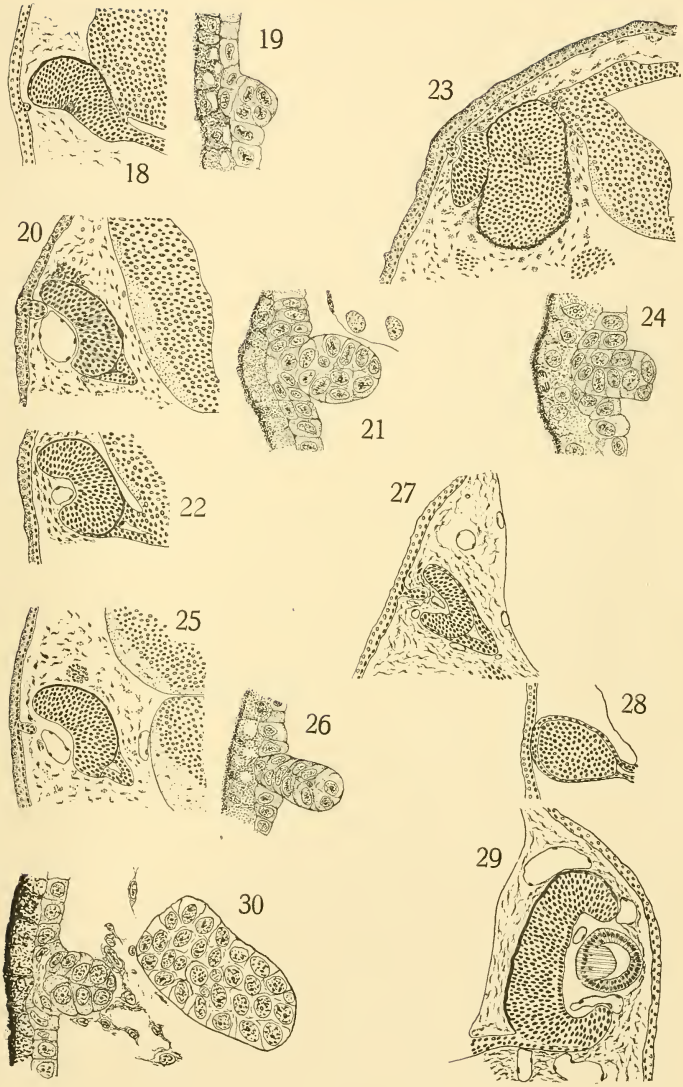

18
19
20
21
22
23
24
25
26
27
28
29
30

FIG. 31. Experiment DF_{43}. Embryo rana palustris killed 4 days after the partial extirpation of the right eye. Section through regenerated right eye and lens-bud. The eye is separated from the ectoderm by mesenchyme. The normal left eye is in outline on the opposite side. × 45 diameters.

FIG. 32. Experiment DF_{43}. Lens-bud more highly magnified. × 360 diameters.

FIG. 33. Experiment DF_{44}. Embryo rana palustris killed 4 days after partial extirpation of the right eye. Section through regenerated eye and lens-bud. The eye is separated from the ectoderm by mesenchyme. The normal eye is similar to the one in Fig. 31. The lens-bud is very similar to that in Fig. 32. × 45 diameters.

FIG. 34. Experiment dx_5. Embryo rana sylvatica killed 4 days after the operation. Section through caudal portion of regenerated eye. Here its outer layer is towards the ectoderm and separated from it by mesenchyme. There is a small lens-bud opposite this portion of the eye. The normal left eye is similar to that of Fig. 29. × 90 diameters.

FIG. 35. Experiment dx_5. Lens-bud more highly magnified. × 360 diameters.

FIG. 36. Experiment DL_{30}. Embryo rana sylvatica killed 5 days after partial extirpation of the right eye. Section through regenerated eye and lens-bud. The regenerated eye is separated from the ectoderm by mesenchyme. There is an artificial separation of the lens-bud from the inner layer of the ectoderm. × 90 diameters.

FIG. 37. Experiment DL_{30}. Lens-bud more highly magnified. × 360 diameters.

FIG. 38. Experiment DL_{30}. Section through normal left eye and lens. × 90 diameters.

FIG. 39. Experiment DF_{79}. Embryo rana palustris killed 5 days after partial extirpation of the right optic vesicle. Section through regenerated right eye showing attachment to brain and separation from ectoderm by mesenchyme. The normal left eye is similar to the one in Fig. 75, experiment DF_{18}. × 90 diameters.

FIG. 40. Experiment DF_{79}. Section through anterior end regenerated eye and small solid lens-sphere which is separate from the ectoderm. The outer layer of the eye is nearest the lens-sphere but separated from it by mesenchyme. × 90 diameters.

FIG. 41. Experiment DF_{79}. Lens-sphere more highly magnified. × 360 diameters.

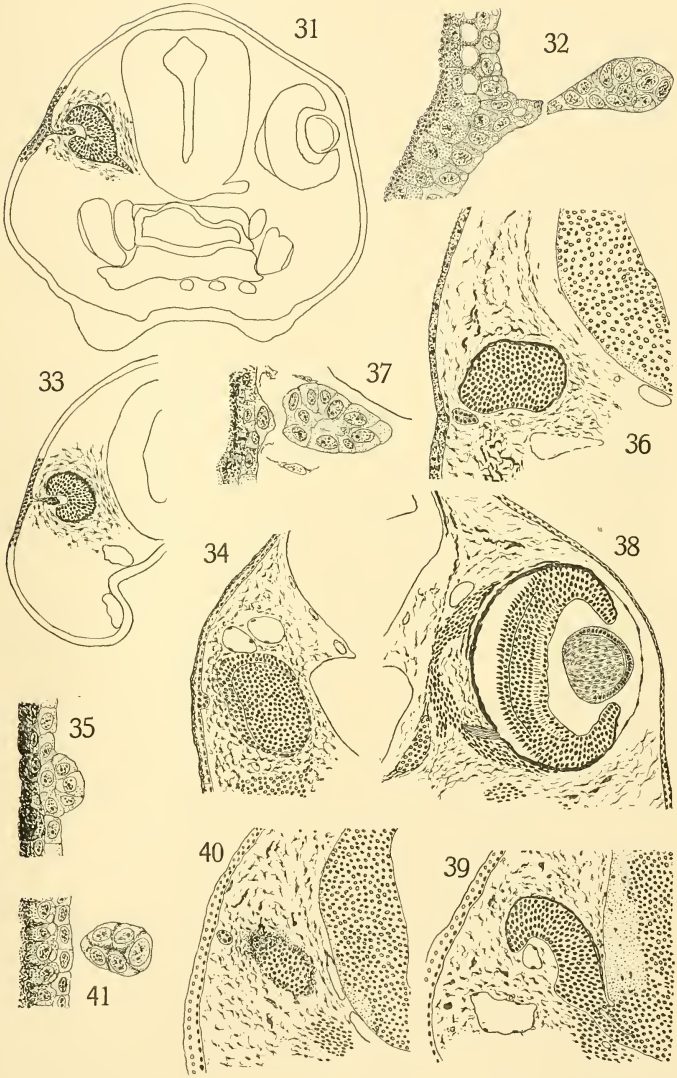

31
32
33
37
36
34
38
35
40
39
41

Fig. 42. Experiment DF_{75}. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Section through regenerated eye and solid lens-sphere. The latter is separate from the ectoderm. × 90 diameters.

Fig. 43. Experiment DF_{75}. Section through normal left eye and lens vesicle. × 90 diameters.

Fig. 44. Experiment DF_{72}. Embryo rana palustris killed 3 days after the operation (see Figs. 20 and 21). Section through edge of normal left eye, edge of lens, lower edge of optic cup and ectoderm showing small lens-bud-like structure attached to the inner layer of the ectoderm. Probably place where lens was pinched off. × 360 diameters.

Fig. 45. Experiment DL_{21}. Embryo rana sylvatica killed 4 days after extirpation and transplantation of the right optic vesicle. Section through lens-like bud of the inner layer of the ectoderm. It is near the otic vesicle but not near either the normal lens area or near the transplanted eye. It was probably caused by injury to the ectoderm during the transplantation of the eye. × 180 diameters.

Fig. 46. Experiment DL_{12}. Embryo rana sylvatica killed 3 days after partial extirpation of right eye. Section through lens-like bud of inner layer of ectoderm, anterior to otic vesicle, and is probably from wound caused in making skin flap. × 180 diameters.

Fig. 47. Experiment DF_{39}. Embryo of rana palustris killed 5 days after partial extirpation of right eye. Regenerated eye has two lenses. Section through lens-like bud of the inner layer, anterior to regenerated eye, and is probably from an injury to the skin flap. × 180 diameters.

Fig. 48. Experiment DL_{28}. Embryo of rana sylvatica killed 4 days after the operation. Section through "lens-like bud" which has arisen between the normal lens-forming region and the otic vesicle about in the region of the wound of incision. × 360 diameters.

Fig. 49. Experiment DL_{34}. Embryo rana sylvatica killed 5 days after the operation. Section through long lens-like bud near otic vesicle. It is probably from injury to the ectoderm made during the transplantation of the eye. × 90 diameters.

Fig. 50. Experiment DL_{24}. Distal end of lens-like bud. × 360 diameters.

Fig. 51. Experiment DL_{10}. Embryo of rana sylvatica killed 3 days after the operation. Section through solid ectodermal spherical mass which has probably arisen from the ectoderm. It is located caudal to the normal lens-forming area about in the region of the wound of incision. × 360 diameters.

Fig. 52. Experiment DL_{26}. Embryo of rana sylvatica killed 5 days after the operation. Section through ectoderm and small lens-like vesicle, located caudal to the normal lens-forming area about in the region of the wound of incision. The ectoderm over the vesicle shows still evidences of the origin of the vesicle and dips down into the mesenchyme. × 360 diameters.

Fig. 53. Experiment DL_{33}. Embryo rana sylvatica killed 5 days after transplantation of optic vesicle into otic region. Section through otic vesicle, transplanted eye, and ventral to it a small lens-like vesicle, probably from injury to the ectoderm. × 90 diameters.

Fig. 54. Experiment DL_{33}. Lens-like vesicle more highly magnified. × 360 diameters.

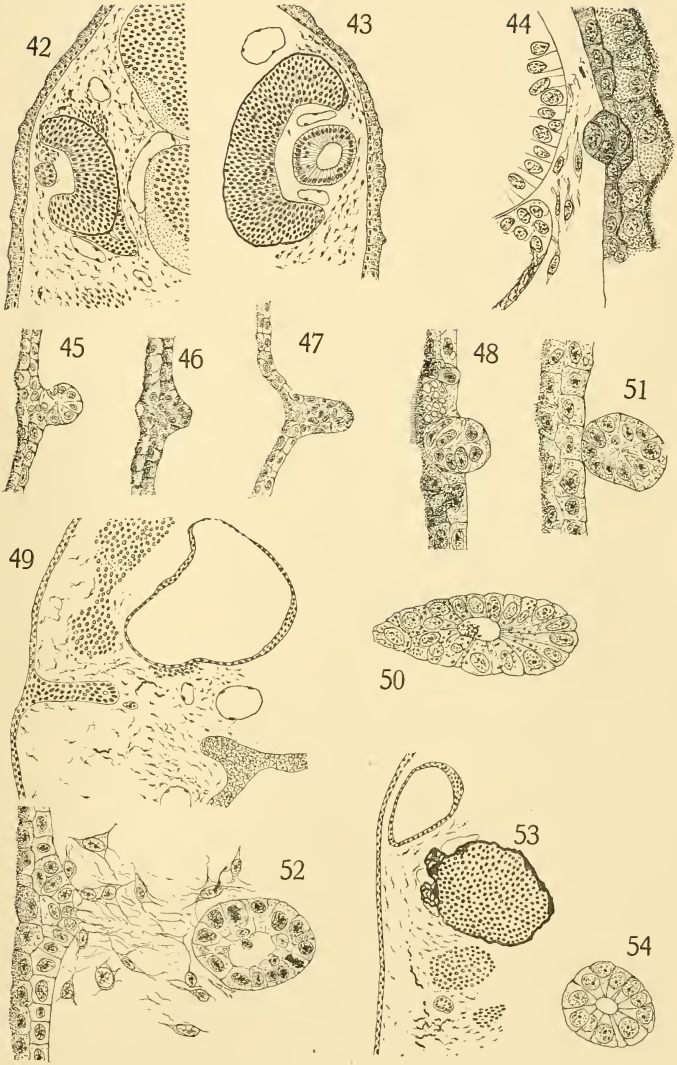

42
43
44
45
46
47
48
51
49
50
52
53
54

FIG. 55. Experiment DL_{59}. Embryo rana sylvatica killed 3 days after transplantation of the optic vesicle into the otic region. Section through edge of outer layer of transplanted eye, ectoderm, and small lens-like vesicle. The transplanted eye has a large lens. × 360 diameters.

FIG. 56. Experiment DL_{43}. Embryo rana sylvatica killed 4 days after transplantation of the optic vesicle into the otic region. Section through ectoderm, anterior end transplanted eye, and lens-like vesicle. The latter is near an injured place in the ectoderm. × 90 diameters.

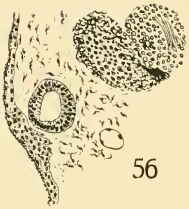
FIG. 57. Experiment DL_{10}. Embryo rana sylvatica killed 3 days after transplantation of the optic vesicle into the otic region. Section through caudal edge of eye and ectoderm showing small lens-like bud. The transplanted eye has a large lens, and this bud is probably an injury process. × 360 diameters.

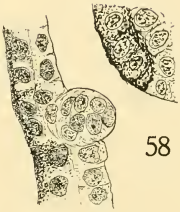
FIG. 58. Experiment DF_{47}. Embryo rana palustris killed 4 days after transplantation of the optic vesicle into the otic region. Section through one edge of the transplanted eye and small lens-bud. The transplanted eye has also another and larger lens. The normal left lens is similar to the one in Fig. 61, experiment DF_{48}. × 360 diameters.

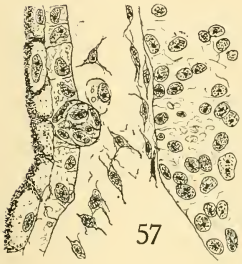
FIG. 59. Experiment DF_{68}. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Transverse section through regenerated and normal eyes, and normal lens vesicle. × 90 diameters.

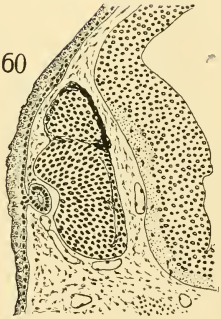
FIG. 60. Experiment DF_{68}. Section caudal to above, through regenerated eye, and end of transplanted eye and small lens-bud. × 90 diameters.


55


56


58


57


60

59

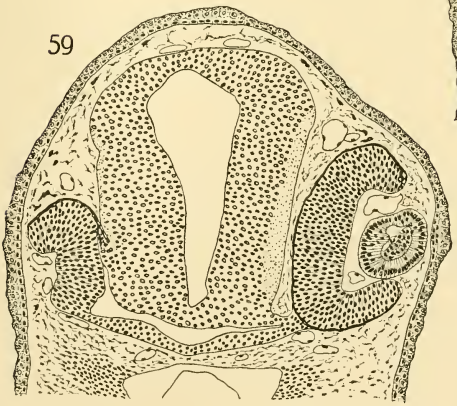


FIG. 61. Experiment DF_{48}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through regenerated and normal eyes. Both regenerated eye and its small lens vesicle are separated from the ectoderm by mesenchyme. The normal side shows the early "lens" stage. × 90 diameters.

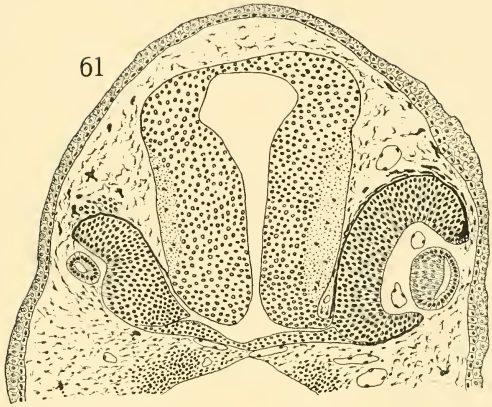
FIG. 62. Experiment DF_{34}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through regenerated eye and small lens vesicle. Normal eye is similar to the one in Fig. 61. × 90 diameters.

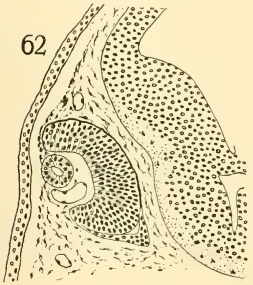
FIG. 63. Experiment DF_{45}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through regenerated eye and small lens vesicle, both are separated from the ectoderm by a layer of mesenchyme. Normal eye as in Fig. 61, experiment DF_{48}. × 90 diameters.

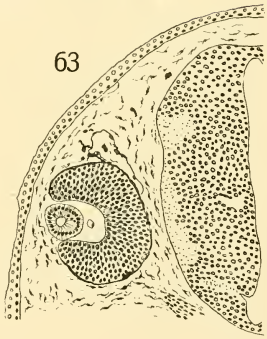
FIG. 64. Experiment DL_6. Embryo rana sylvatica killed 3 days after partial extirpation of the right optic vesicle. Section through regenerated and normal eyes. × 45 diameters.

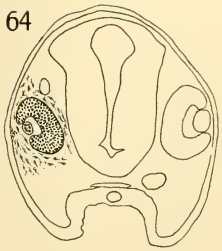
FIG. 65. Experiment DL_6. Section through small lens vesicle of regenerated eye. × 180 diameters.

FIG. 66. Experiment DL_6. Section through normal lens vesicle. × 180 diameters.


61


62


63


64


65

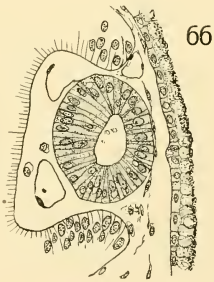

66

Fig. 67. Experiment DF_1. Embryo rana palustris killed 3 days after partial extirpation of the right optic vesicle. Section through large regenerated eye and lens-bud. × 90 diameters.

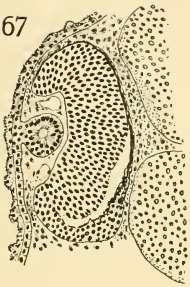
Fig. 68. Experiment DF_1. Section through normal eye and lens vesicle. × 90 diameters.

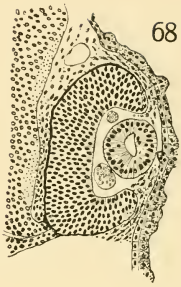
Fig. 69. Experiment DF_{10}. Embryo rana palustris killed 3½ days after partial extirpation of the right optic vesicle. Section through large regenerated eye and lens vesicle. × 90 diameters.

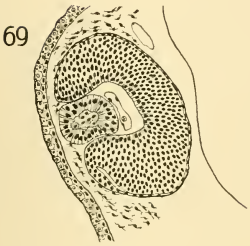
Fig. 70. Experiment DF_{10}. Section through normal eye and lens. × 90 diameters.

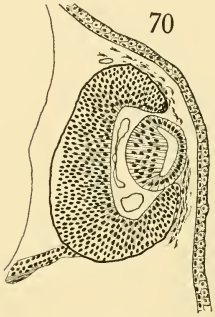
Fig. 71. Experiment DF_{61}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through regenerated eye and small lens. Normal eye, as in Fig. 77.

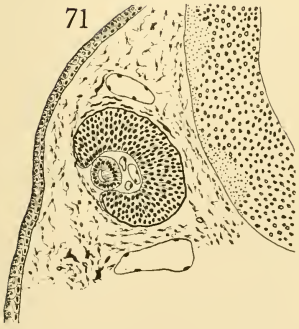
Fig. 72. Experiment DF_{40}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through the regenerated right eye and small lens, both are separated by a considerable layer of mesenchyme from the ectoderm. Normal eye, as in Fig. 77. × 90 diameters.


67


68


69


70


71

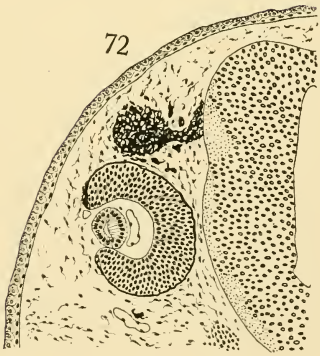

72

Fig. 73. Experiment DF_{65}. Embryo rana palustris killed 5 days after partial extirpation of the right optic vesicle. Section through regenerated eye and lens. Normal eye, as in Fig. 75. × 90 diameters.

Fig. 74. Experiment DF_{18}. Embryo rana palustris killed 5 days after partial extirpation of the right optic vesicle. Section through large regenerated eye and lens. × 90 diameters.

Fig. 75. Experiment DF_{18}. Section through normal eye and lens. × 90 diameters.

Fig. 76. Experiment DF_{63}. Embryo rana palustris killed 4 days after partial extirpation of the right optic vesicle. Section through large regenerated eye and lens. × 90 diameters.

Fig. 77. Experiment DF_{63}. Section through normal left eye and lens. × 90 diameters.

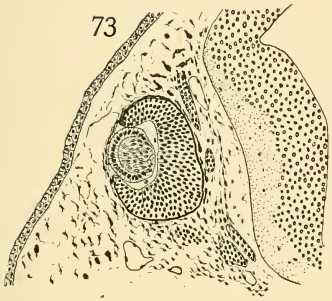
Fig. 78. Experiment DF_{46}. Section through normal eye of rana palustris killed 4½ days after the operating stage. × 90 diameters.

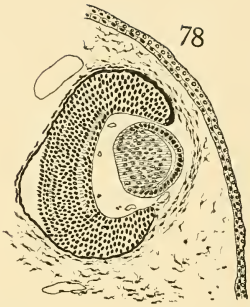
Fig. 79. Experiment DL_{18}. Embryo rana sylvatica killed 4 days after operation on the right optic vesicle. Section through degenerating left eye and small lens vesicle. A normal eye at this age is as in Fig. 77. × 90 diameters.

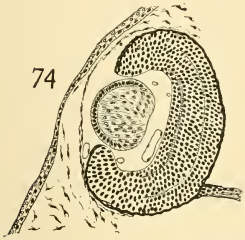
Fig. 80. Experiment DL_{29}. Embryo rana sylvatica killed 4 days after operation on the right optic vesicle. Section through degenerating left eye and small lens vesicle, compare with a normal eye at this stage, Fig. 77. × 90 diameters.

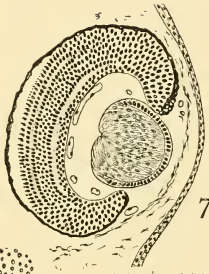
Fig. 81. Experiment DL_{19}. Embryo rana sylvatica killed 4 days after operation on the right optic vesicle. Section through degenerating left eye and small lens vesicle. Normal eye of an embryo at this age, as in Fig. 77. × 90 diameters.

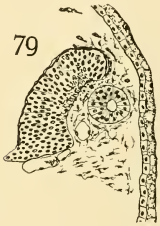
Fig. 82. Experiment DL_{19}. Section through more caudal part of same eye as in Fig. 81, showing second small lens vesicle which is entirely separate from the first. × 90 diameters.

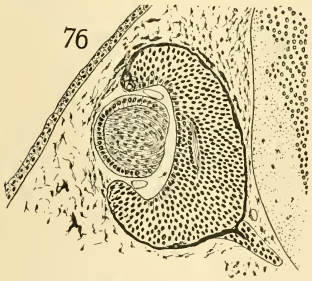

73

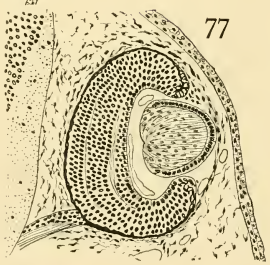

78


74

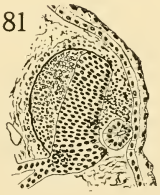

75

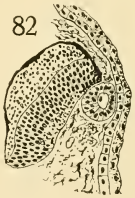

79


76


77


80


81


82

THE EMBRYONIC HISTORY OF THE LENS IN BDELLOSTOMA STOUTI IN RELATION TO RECENT EXPERIMENTS.

BY

CHARLES R. STOCKARD.

Pathological Laboratory, Cornell University Medical College, New York City.

WITH 3 TEXT-FIGURES.

Spemann,[1] Lewis,[2] and others, have shown by experiments on amphibian embryos that there is no localization of lens-forming material in any given area of the ectoderm, and that the formation of a lens depends directly upon the stimulation of the ectoderm by a contact with the optic-cup. Spemann [3] has since discussed the question of the self-differentiating power of the lens and concluded from a consideration of Schaper's [4] experiments on the frog that the lens is not self-differentiating, but that a durable influence or contact of the optic-cup is necessary to cause the lens-plate or lens-bud to develop into a typical lens. Le Cron [5] has lately shown by a series of convincing experiments that the lens in Amblystoma is not self-differentiating. He found when the optic-cup was artificially removed from below the lens-plate, lens-bud, or lens-vesicle that the lens structure soon ceased to further differentiate and commenced to undergo degeneration. In most of these experiments the authors have considered the possibility that the injury caused by the operation might be responsible for the failure of the lens to form, although their methods and care have been sufficient to convince one that such was not the case.

[1] Spemann, H., Ueber Correlationen in der Entwickelung des Auges. Verhandl. der Anat. Gesellsch., 1901.

[2] Lewis, W. H., Experimental Studies on the Development of the Eye in Amphibia. I. On the Origin of the Lens. Rana palustris. Am. Jour. Anat., III, 1904.

[3] Spemann, H., Ueber Linsenbildung nach experimenteller Entfernung der primären Linsenbildungzellen. Ausführlich: Zoöl. Anz., 28, 1905.

[4] Schaper, A., Ueber einige Fälle atypischer Linsenentwickelung unter abnormen Bedingungen. Anat. Anz., XXIV, 1904.

[5] Le Cron, W. L., Experiments on the Origin and Differentiation of the Lens in Amblystoma. Am. Jour. Anat., VI, 1907.

In the blind fishes, however, we have normal cases of degeneration of the eye structures which one might expect to at least partially analyze with the aid of the experimental results. Eigenmann[6] has shown in Amblyopsis, a blind cave fish, that a lens structure is present in young embryos and soon disappears, being entirely absent in old embryos. No connection was noted between the appearance and disappearance of the lens and the contact of the optic-cup with the ectoderm, although the significance of such a relation is only made clear by the experiments. In the adult eye of the burrowing lizard, Rhineura of Florida, Eigenmann recorded that the lens was absent in one-half of the eyes studied, while the organ was extremely variable in those eyes in which it was found.

Müller,[7] in his early description of the Myxinoids, and lately Allen,[8] in studying the eye of the adult Bdellostoma noted the absence of a lens. Miss Worthington,[9] observing the living animals, records them to be totally blind.

Price,[10] the first to study the embryos of Bdellostoma, found in a young stage that a projection of cells from the inner layer of the ectoderm extended toward the optic-cup. In older embryos this structure, which Price interpreted correctly to be the lens-bud, had disappeared. Kupffer[11] shows a slight thickening of ectoderm in one of his figures and designates it a lens-placode; this he states disappears in older embryos. In a former paper I[12] mentioned the disappearance of the lens in the embryos of Bdellostoma. It is thus seen that the development of this lens has received only passing notice, while in the light of experiments the case seems to have gained sufficient importance to warrant a fuller description.

While studying the development of the brain and special sense organs

[6] Eigenmann, C. H., The History of the Eye in Amblyopsis. Proc. Indiana Acad. Sci., 1901.

[7] Müller, J., Vergleichende Anatomie der Myxinoiden, der Cyclostomen mit durchbohrtem Gaumen. Berlin, 1839.

[8] Allen, B. M., The Eye of Bdellostoma Stouti. Anat. Anz., XXVI, 1905.

[9] Worthington, J., Contributions to Our Knowledge of the Myxinoids. Am. Nat., XXXIX, 1905.

[10] Price, G. C., Some Points in the Development of a Myxinoid. Verhandl. der Anat. Gesellsch., 1896.

[11] Kupffer, C., Zur Kopfentwicklung von Bdellostoma. Sitzungsberichten d. Gesellsch. f. Morph. u. Physl., München, 1900.

[12] Stockard, C. R., The Development of the Mouth and Gills in Bdellostoma Stouti. Am. Jour. Anat., V, 1906.

in Bdellostoma I have been impressed with the manner in which the history of the lens in these embryos seems to corroborate the conclusions drawn by the experimenters mentioned above. In a brief way I wish to present these points, which are readily interpreted in the light of the

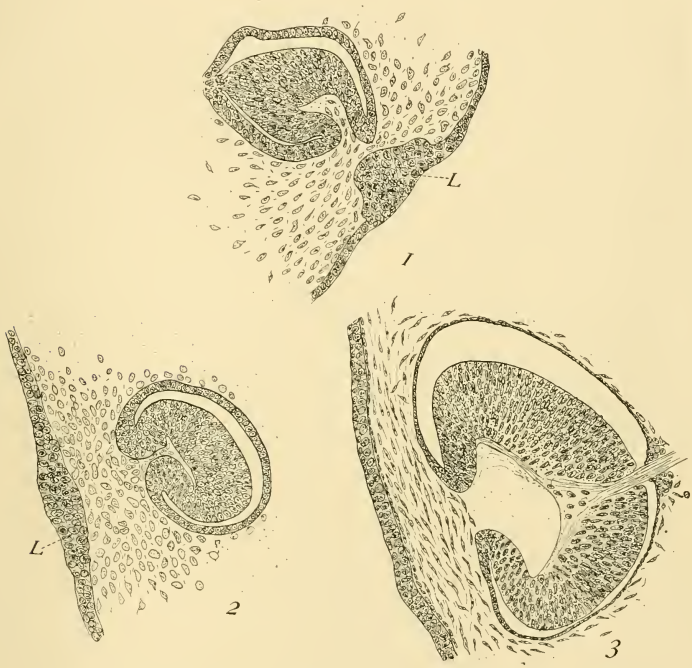


Fig. 1. A section through the eye of a 15 mm. embryo of Bdellostoma. *L*, the lens at the height of its development, the contact between the optic-cup and the ectoderm has just been lost.

Fig. 2. The eye of an older embryo, the lens, *L*, degenerating and the optic-cup well removed from the outer wall.

Fig. 3. The eye of an old embryo in which the lens has entirely disappeared. All camera drawings to the same scale.

experiments, while they in turn also lend support to the experimental conclusions by showing that many of the conditions artificially produced may occur in a normal embryo.

Very early embryos of Bdellostoma in which the nose is still a single tube, and in which six or seven gill slits are present on the laterally outspread plates, will show the lens in the following condition: A small antero-dorsal portion of the irregularly shaped optic-cup comes in contact with the ectodermal head-wall, and from this ectoderm a projection of cells extends inward toward the cavity of the optic-cup. The lens-bud is thus to an extent conical in form and *results from a contact of only a portion of the optic-cup with the ectoderm.* This structure continues to develop for a time until in an embryo considerably more advanced and measuring 15 mm. in length one sees the lens-bud with a slight indication of a constriction about the periphery of its area of union with the ectoderm, as if it were preparing to pinch off (Fig. 1). Here the progressive development of the lens ceases and degeneration begins. At this stage also the contact of the optic-cup with the ectoderm or lens-bud is just being lost.

An older embryo in which all of the gill clefts have appeared, but are still on the outspread lateral plates, and in which the nose exists as two parallel tubes, shows the lens much reduced in extent. The optic-cup is now well separated from the ectodermal wall and a considerable layer of mesenchymous tissue is seen between the two (Fig. 2). The lens, *L,* here is indicated only by a slightly thicker area of ectoderm over the deeply buried optic-cup. In all embryos older than this one no indication whatever of a lens-like thickening could be found, the ectoderm over the eye region being of the same thickness as that of adjacent areas (Fig. 3). This figure also shows that the optic-cup has continued to differentiate its parts, and is probably not so degenerate as to be unable to influence the ectoderm should it remain in contact with it.

It is thus shown that the lens is not normally self-differentiating but begins to degenerate when contact with the optic-cup is lost.

The embryos of Bdellostoma illustrate, therefore, by the changes which their lenses undergo many of the points sought in the above-mentioned experiments. They clearly show that *the lens formation is directly dependent upon a contact of the optic-cup with the ectoderm. Secondly, contact with only a portion of the optic-cup is necessary to cause the ectoderm to begin lens formation. Thirdly, to produce a lens the contact of the optic-cup with the ectoderm must be durable; and fourthly, the optic-vesicle may change into an optic-cup without the aid of the mechanical pressure of the lens.* This series of events would be difficult to interpret without the facts demonstrated by the experiments, while on the other hand it adds strength to the conclusions drawn

from the experiments by proving that such results may occur under normal conditions and are, therefore, in no way attributable to the injury caused by the operations.

The question of the localization of lens-forming material in a given ectodermal area is not answered by Bdellostoma, but I [13] have shown in artificially produced cyclopean monsters in the Teleost that a lens may form from a region out of the usual lens-forming area, and that the size of the optic-cup regulates the size of the lens. So, in other fishes, just as in amphibians, we would not expect to find localized lens-forming regions in the ectoderm.

I wish to express my indebtedness to Professor Bashford Dean for kindly placing at my disposal his complete series of Bdellostoma embryos.

[13] Stockard, C. R., The Artificial Production of a Single Median Cyclopean Eye in the Fish Embryo by Means of Sea-Water Solutions of Magnesium Chlorid. Arch. Entw.-Mech., XXIII, 1907.

INDEX TO VOL. VI.

(See also Contents of this Volume for Full Titles and Authors' Names.)

References to articles appearing in THE ANATOMICAL RECORD will be indexed separately on the completion of every 300 pages.