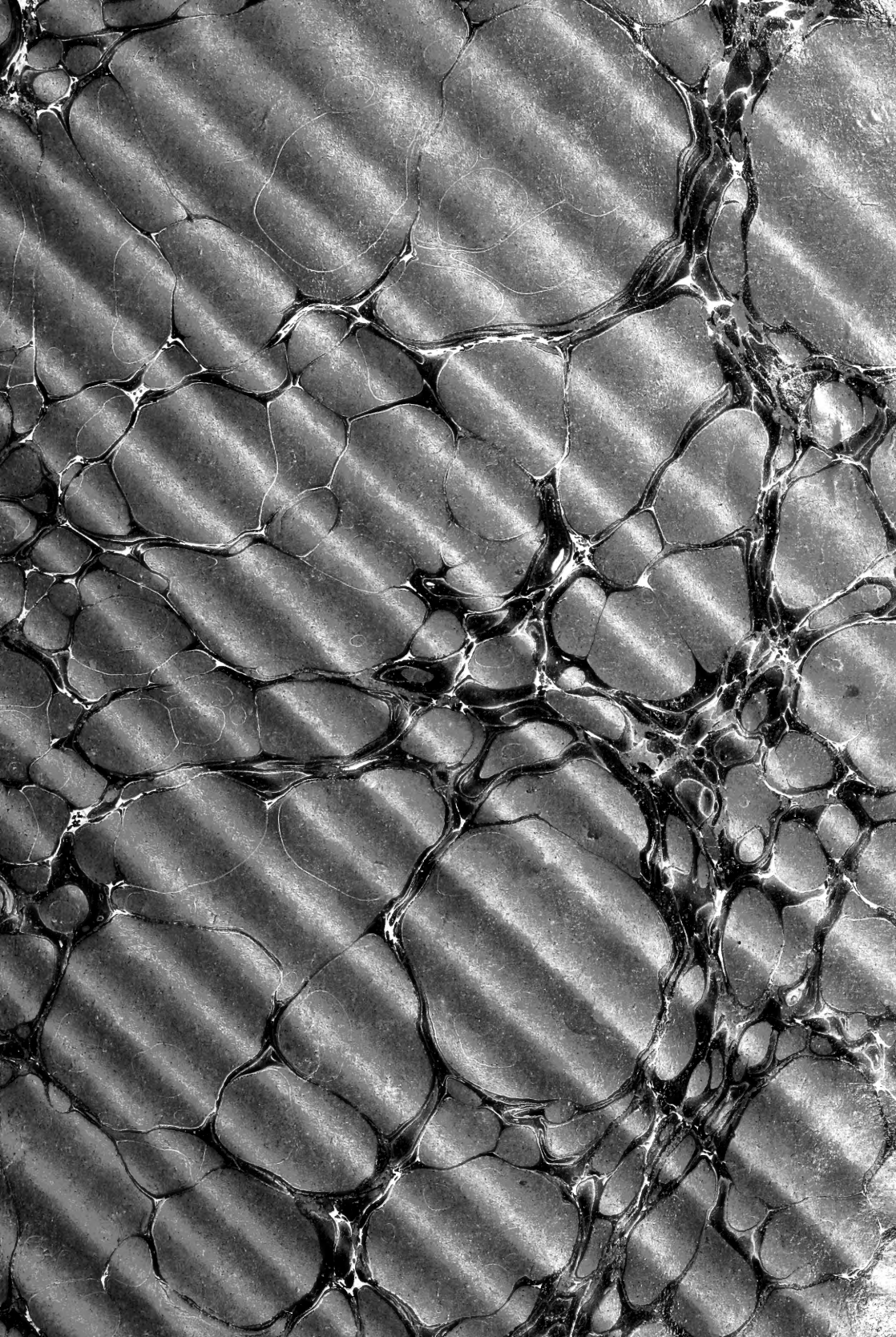
This pelt included the first 2 vols of the Journal of Morphology

This pelt was ripped and used by Mrs. Kaan & Miss Coy.



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CONTENTS OF VOL. I

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THE AMERICAN JOURNAL OF ANATOMY.

THE AMERICAN JOURNAL OF ANATOMY has been founded to collect into one place, and present in a worthy manner, the many researches from our investigators, now scattered through many publications at home and abroad.

Human Anatomy in America needs as high a standard of reference as it has in other countries. Without such a standard it fails to make for itself any proper, satisfactory or stimulating impression. The best interests of modern scientific medicine will be greatly advanced by the upholding of such a standard in this fundamental subject through a journal of high character.

Many aspects of Comparative Anatomy, Embryology, Histology and Cytology are so intimately bound up with the problems of Human Anatomy that these subjects will be included within the scope of the new journal. It will be the aim of THE AMERICAN JOURNAL OF ANATOMY to recognize this close natural relationship between the various branches of the science, and to publish results of the best original work of American students of anatomy.

The most cordial assurance of support has been given by the collaborators, and this we believe is sufficient indication of the results to be expected.

A number of generous persons, whose names will appear later, have given some financial support to help us in gaining a foothold in a suitable manner. The journal must however look to those who are to be benefited by its publication for its real and permanent support; and a good list of regular subscribers is expected and required to maintain it.

It is hoped that those interested in promoting a worthy development of research in America, in the subjects included within the scope of this journal, will energetically assist us.

Henry E. Crampton

DEVELOPMENT OF THE LIMBS, BODY-WALL AND BACK IN MAN.

BY

CHARLES RUSSELL BARDEEN, M. D. AND WARREN HARMON LEWIS, M. D.

From the Anatomical Laboratory of the Johns Hopkins University, Baltimore, Md.

WITH 9 PLATES AND 27 TEXT FIGURES.

The purpose of the following paper is a description of various typical stages in the development of the back, the limbs, and the body-wall in man. The work is based primarily upon reconstructions, according to the method of Born,[1] of parts of five human embryos; it has been extended and controlled by a study of the external form and of serial sections of several other human embryos. Dr. Lewis has devoted special study to the formation of the arm, Dr. Bardeen to that of the leg, the body-wall and the back.

In the accompanying table a list is given of the embryos utilized. Those marked with an asterisk have been reconstructed.

We shall consider the early stages in the development of the limbs, the body-wall and the back, first, from the point of view of the external form and, secondly, from that of internal structural differentiation.

I. EXTERNAL FORM.

The external form of the embryos we have used has been compared with that of embryos of a corresponding stage of development pictured in the His Atlas.[2] Figs. 1-15, on pages 3 to 9 represent a series of

ERRATUM FOR VOL. I, NO. 1.

Vol. I, No. 1, page 83, Fig. 2, of this Journal should read:

FIG. 2. Embryo of 13.0 mm. Sagittal series, No. 224, section 65. ×30 diams.

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[1] See Bardeen: Wax plate reconstruction according to the method of Born as utilized in the Anatomical Laboratory of the Johns Hopkins University. The Johns Hopkins Bulletin, April-May-June, 1901.

[2] Anatomie menschlicher Embryonen, Leipzig, 1885.

[3] The ages given are for the most part only roughly approximate.

TABLE I

EMBRYOS STUDIED.

	Length in mm.	Probable age in weeks.	Size of Ovum in mm.	Thickness of section in micro mm	Number of Myotomes.	Myotomes opposite the arm	Myotomes opposite the leg	No. of Myotomes between the arm and leg regions
*XII	2.1	2	18x18x18	10	3o. 14 8c. 3t.	5c–1t	1l–1s	11
LXXVI	4.5	3	22x20	20	3o. 5s. 35 8c. 2-3c. or 12t. 36 5l.	5c–1t	1l–1s	11
CXLVIII	4.3	3	17x14x10	10	2o. 2s. 8c. 27 10t. 5l.	7–11 5c–1t	21–25 or 26 1l–1s	9
LXXX	NB 5 VB 4.5	3	24x18x18	20	cannot be counted with certainty.			
*II	NB 7 VB 6	4	25x25	15	3o. 5s. 8c. 5-6c. 38 12t. 5l.	5c–1t	1l–1s	11
*CLXIII	NB 9 VB 9	4½	35x35x20	20	8c. 5s. 34 12t. 4c. 5l.	4c–1t	1l–1 or 2s	11
*CIX	NB 10.5 VB 11	5	30	20	Myotomes have disappeared.			
CXLIV	NB 12 VB 14	5½	40x30x30	40				
CLXXV	NB 13 VB 13	5½	30	uncut				
CVI	NB 15.5 VB 17	5½		50				
CLXVII	NB 14.5 VB 13.5	5½	33x30x20	uncut				
*XLIII	NB 14 VB 16	6		50				
*XXII	NB 18 VB 20	7	35x30x30	50				

The Roman numerals refer to embryos in the collection of human embryos belonging to Prof. Mall, in the Anatomical Laboratory of the Johns Hopkins University. To Dr. Mall we are greatly indebted for the use of these embryos.

Reference to the embryos given in Table I will be found in the following articles by Dr. Franklin P. Mall. No. II, A Human Embryo Twenty-six Days Old, Jour. of Morph., Vol. V; Nos. II, XII, XXII and XLIII, Development of the Human Coelom, Jour. of Morph., Vol. XII; Nos. II, XII and XXII, Ueber die Entwickelung des Menschlichen Darmes, Arch. für Anat. und Phys., Special Bd., 1897; Nos. II, XLIII and LXXVI, Development of the Internal Mammary and Deep Epigastric Arteries in Man, Johns Hopkins Hospital Bulletin, 1898; Nos. II, XII, XXII, XLIII, LXXVI and CIX, Development of the Ventral Abdominal Walls in Man, Jour. of Morph., Vol. XIV, 1898; Nos. II, XII, XXII, LXXX, CVI, CIX and CXLVIII, A Contribution to the Study of Pathology of Early Human Embryos, Johns Hopkins Hospital Reports, Vol. IX, 1901.

sketches of the embryos indicated. On Plate I the photographs utilized are reproduced. On Plates II to IX are represented several typical stages in the general development of the body-wall and limbs. Figs. A and B, Plate II, are drawn from wax-plate reconstructions. Figs. C to E, Plates III to V, are based, in the main, upon a reconstruction of the regions of the arm, abdomen and leg of embryos CLXIII and CIX, and upon excellent photographs. Figs. F to I, Plates VI to IX, are based upon wax-plate reconstructions of Embryo XXII.

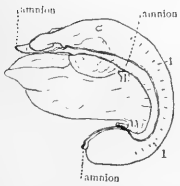


Fig. 1.
x about 10 d.

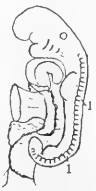


Fig. 2.

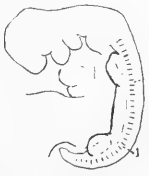


Fig. 3.

Fig. 4.

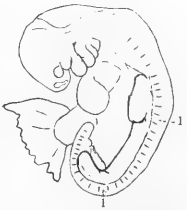


Fig. 5.
x about 5 d.

Embryo XII.

The development of the neuro-muscular apparatus begins in the human embryo in the cervical region. In Fig. 1 is represented Embryo XII, 2.1 mm. in length and about two weeks of age. The axis of the embryo is curved in a semicircle about the heart and the umbilical vesicle. The axis contains neural tube, notochord, myotomes, dorsal-aortæ, and mesenchyme (see Fig. 16). There are fourteen myotomes on each side. Mall considers three of them occipital, eight cervical, and three thoracic. The first cervical and the first thoracic myotomes are numbered "1" in Fig. 1. Caudal to the fourteenth myotome, an unsegmented band of tissue extends along each side of the spinal cord. The neural tube is open dorsally anterior to the fourth and posterior to the fourteenth myotome. Opposite the twelfth myotome a solid band of cells, the "neurenteric canal," unites spinal-cord and entoderm. The notochord extends from a point opposite the cephalic margin of the heart to the region of the neurenteric canal. The dorsal aortæ run a course parallel with the notochord, but extend further than the myotomes caudally. A considerable amount of mesenchyme is formed at the cephalic extremity of the axis of the body, in the region of the heart, but toward the caudal extremity little exists. The heart and the pericardial and pleural cavities are developed in the cephalic region of the wall of the umbilical vesicle. Between the region of the heart and the neural

tube the pharynx extends forwards. From it project the first and second branchial pockets, Seessel's pocket, and the thyroid diverticulum. Into the caudal end of the embryo the hind-gut extends. The umbilical vesicle projects forwards from the region opposite the 1-6 cervical myotomes (see Fig. 1). At this period the amnion arises on each side along the length of the axis of the embryo as far forward as the region of the heart (Fig. 1). Externally and internally the amnion is covered by a layer of epithelial cells. From epithelium lining the cœlom, several layers of cells have arisen (Fig. 16). There is, however, as yet, no true body-wall caudal to the region of the heart. There are no external visible signs of limb buds.

Embryo Lr.

In Fig. 2 is represented the His embryo Lr; length, neck-breach, 4.2 mm.; age, about three weeks. The back of this embryo presents a slight concavity opposite the ninth (first thoracic) myotome. It is probable that this is an artifact, due to the removal of the embryo from the ovum, and that in the natural condition the back curved about the viscera as it does in the embryos represented in Figs. 1, 3, 4 and 5.[4] "Lr" shows externally thirty-one myotomes (8c, 12t, 5l, 5s, 1c). The ninth (first thoracic) and twenty-first (first lumbar) myotomes are designated by the numeral "1." Lateral to the region of the myotomes lies the Wolffian ridge, a band of tissue which represents the anlage of the limbs and body-wall. The arm is represented by a slight swelling opposite the 5th to 8th cervical and 1st thoracic myotomes. The leg is represented by a slight swelling opposite the 1st to 5th lumbar and 1st sacral myotomes. The amnion was probably attached, in this embryo, to the umbilical cord. Between the Wolffian ridge and the umbilical cord the *membrana reuniens* extends, at this period, so as to cover over the thoracic and abdominal viscera. It is represented as torn along the heavy irregular line.

Embryo CXLVIII.

In Fig. 3 is represented Embryo CXLVIII; length, neck-breach, 4.3 mm.; age, about three weeks. A photograph of the embryo is given on Plate I. Though more advanced in development than Lr, but twenty-seven myotomes are present (2o, 8c, 10t, 5l, 2s). This has been determined by careful counting of the myotomes in serial sections of the embryo. The base of the arm-bud appears to lie opposite the sev-

[4] See Mall, Human Cœlom, op. cit., p. 421.

enth to the eleventh myotomes. It is, therefore, probable that two occipital myotomes are present. But nine myotomes lie in the area between the arm-bud and the leg-bud. The base of the latter lies opposite 21st to the 25th or 26th myotomes. If two myotomes be considered occipital myotomes, the leg, in this instance, lies two segments nearer the head than usual. It is therefore probable that this embryo has an unusually short body-wall.

Embryo LXXVI (length, 4.5 mm.; age, about three weeks) is of essentially the same stage of development as CXLVIII. It has thirty-five myotomes (3o, 8c, 12t, 5l, 5s, 2c). The base of the arm lies opposite the eighth (fifth cervical) to the twelfth (first thoracic) myotomes. The base of the leg lies opposite the twenty-fourth (first lumbar) to the twenty-ninth (first sacral) myotomes. Eleven myotomes lie between the regions of the arm and leg-buds. In CXLVIII the limb-buds protrude more than in LXXVI and the body-wall extends further ventrally.

Embryo LXXX (length, 5 mm.; age, about three weeks), a photograph of which is given in Plate I, is similar, though slightly more advanced in development than Embryo CXLVIII.

Embryo .

In Fig. 4 is represented the His embryo α; length, neck-breach, 4 mm.; age, about 23 days. The back of this embryo is very greatly flexed. Thirty-five myotomes are present (8c, 12t, 5l, 5s, 5c). The arm-bud lies opposite the 5th to 8th cervical and 1st thoracic myotomes; the leg-bud opposite the 1st to 5th lumbar and 1st sacral myotomes. Both protrude further than in CXLVIII. The arm-bud projects in a caudal direction. The leg is represented, not as in the original His drawing, but instead in the more normal position shown in the His drawing of the right side of the same embryo.

Embryo R.

In Fig. 5 is represented the His embryo R; length, 5 mm.; age, about 3½ weeks. Thirty-five myotomes are pictured (8c, 12t, 5l, 5s, 5c). The arm-bud lies opposite the 5th to 8th cervical and 1st thoracic myotomes; the leg-bud opposite the 1st to 5th lumbar and 1st sacral. Both point somewhat caudally.

Embryo II.

In Fig. 6 is represented Embryo II; length, neck-breach, 7 mm.; vertex-breach, 6 mm.; age, about 4 weeks. Thirty-eight myotomes are

present (3o, 8c, 12t, 5l, 5s, 5-6c). The extensions of the myotomes within the body wall are pictured. The base of the arm-bud lies opposite the 5th to 8th cervical and 1st thoracic myotomes; that of the leg-bud opposite the 1st to 5th lumbar and 1st sacral myotomes. The arm-bud projects caudally, the leg-bud outwards and slightly caudally.

Embryo A.

In Fig. 7 is represented the His embryo A; length, 7.5 mm.; age, about 4 weeks. Thirty-five myotomes are pictured (8c, 12t, 5l, 5s, 5c). The arm-bud lies opposite the 5th to 8th cervical and 1st thoracic; the leg-bud lies opposite the 1st to 5th lumbar and 1st sacral myotomes.[5] Both project caudally. Both show a slight division into segments. This, however, is much more marked in the following embryo.

Fig. 6.

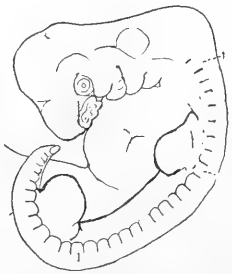


Fig. 7.

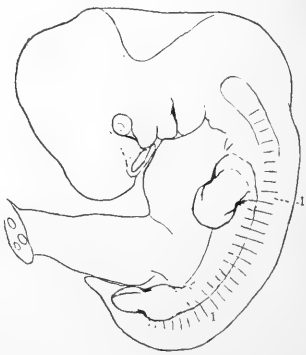


Fig. 8.

x about 5 d.

Embryo CLXIII.

In Fig. 8 is represented Embryo CLXIII; length, 9 mm.; age, about $4\frac{1}{2}$ weeks. Two photographs of this embryo are shown on Plate I. Thirty-three myotomes are present (8c, 12t, 5l, 5s, 3c). The base of the arm lies opposite the 4th to 8th cervical and 1st thoracic, and that of the leg opposite the 1st to 5th lumbar and 1st to 2nd sacral myotomes. The arm projects nearly caudally. A constriction on the cephalic and caudal borders separates the rounded upper arm from the flattened lower arm and hand. The constriction on the caudal border is close to where the arm joins the body-wall, while that on the cephalic border is at a point some distance from the body-wall. This difference on the two borders is to be correlated with the caudal projection of the arm.

[5] This statement is based on the drawing given in Fig. 2, Plate I* of the Atlas.

The medio-lateral flattening of the distal portion of the arm-bud is especially well marked. Proximal to this flattened portion swellings on both medial and lateral surfaces indicate where pre-muscle tissue is developed. A constriction may likewise be seen dividing the leg-bud into two distinct divisions. Owing to a slight torsion the lower portion of the leg-bud presents to view the anterior margin instead of the flattened lateral surface.

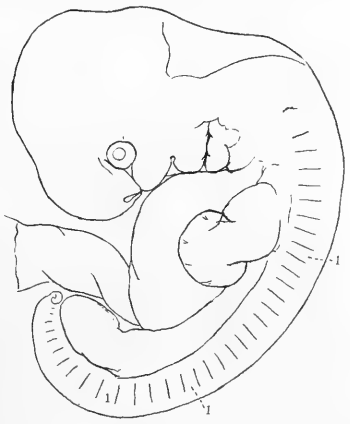


Fig. 9.

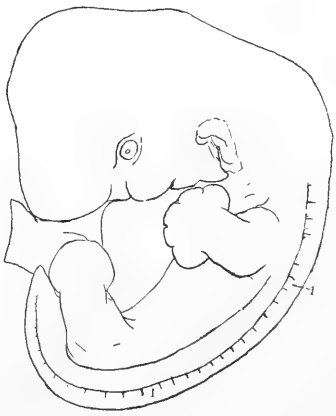


Fig. 10.

x about 5 d.

Embryo Br_1.

In Fig. 9 is represented the His embryo Br_1; length, 11 mm.; age, about $4\frac{1}{2}$ weeks. Thirty-five myotomes are pictured (8c, 12t, 5l, 5s, 5c).[6] The base of the arm lies opposite the 4th to 8th cervical and 1st thoracic spinal ganglia. The division of the arm into its main segments is advanced beyond that pictured in Embryo CLXIII. The upper arm still projects caudally. The lower arm, owing to flexion at the elbow, projects caudo-ventrally. The hand is flattened and can be distinguished from the forearm. Swellings of the digits are visible. The first indications of the shoulder are present. The posterior limb shows a differentiation of foot, leg and thigh regions.

Embryo CIX.

Fig. 10 represents Embryo CIX; length, 11 mm.; age, about 5 weeks. Two photographs of this embryo are reproduced in Plate I. The base

[6] Externally visible segmentation at this stage is due to the spinal ganglia, not to myotomes. The latter have lost their identity.

of the arm lies opposite the 3d to 8th cervical and 1st thoracic spinal ganglia. The upper arm still projects caudally. The forearm is more flexed and projects ventrally; it is now quite well marked off from the upper arm and hand. The digital swellings have increased and are now visible on the margin as well as on the flattened surface of the hand. The shoulder is more marked. The base of the arm is larger and extends higher in the cervical region than in the younger stages. The posterior limb shows a distinct differentiation of the foot. The knee-bend may be distinguished. The hip region is not clearly marked externally.

Fig. 11. Fig. 12. Fig. 13.
x about 2½ d.

Embryo CLXXV.

Fig. 11 represents Embryo CLXXV; length, 13 mm.; age, about 5½ weeks. Two photographs of this embryo are given in Plate I. The various regions of the arm and the swellings of the digits are well marked. The forearm has a more caudal projection than in Fig. 10. In the posterior limb the various regions are more or less distinctly indicated. In the foot digitation has begun. The body wall has advanced half-way across the surface of the liver.

Embryo CVI.

Fig. 12 represents Embryo CVI; length, vertex-breach, 17 mm., neck-breach, 15.5 mm.; age, about 5½ weeks. A photograph is reproduced on Plate I. The limbs and body-wall are similar in development to those of Embryo CLXXV, but the flexion at the elbow and knee is more marked, and the body-wall has advanced further across the abdomen.

Embryo CLXVII.

Fig. 13 represents Embryo CLXVII; length vertex-breach 14.5 mm., neck-breach 13.5 mm.; age, 5½ weeks. A photograph is shown on

Plate I. While the embryo is similar in general differentiation to Embryos CLXXV (Fig. 11) and CVI (Fig. 12), the development of the digits of the hands and feet is further advanced. The flexion of the forearm is also more marked.

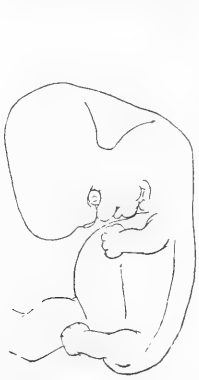


Fig. 14.

Fig. 15.

x about 2½ d.

Embryo XLIII.

Fig. 14 represents Embryo XLIII; length, vertex-breach 16 mm., neck-breach 14 mm.; age, about 6 weeks. The forearm and leg have grown considerably beyond the stage shown in Figs. 11-13, but without marked alteration in external form.

Embryo XXII.

Fig. 15 represents Embryo XXII; length, vertex-breach 20 mm., neck-breach 18 mm.; age, about 7 weeks. A photograph is reproduced on Plate I. The limbs have begun to resemble those of the adult. In the hand the clefts between the digits are well marked. The forearm and hand are somewhat pronated. The tips of the digits now reach nearly to the ventral mid-line. In the hind-limb the foot still lies in the same plane with the leg. The twist at the ankle which brings the foot into adult position has not begun. The toes are fairly distinct.[7]

[7] A number of embryos have been pictured which correspond essentially in external form to those above described. The following list gives reference to the articles in which several of these have been described and pictured.

To the His embryo R, Fig. 5, corresponds:

Foll's 5.6 mm. embryo, Fig. 217, p. 386, Minot's Embryology.

To No. II, Fig. 6, corresponds:

Keibel's embryo H. S., length 8 mm., Arch. für Anat. und Phys., 1891, Taf. XIX, Fig. 2.

RELATIONS OF THE LIMBS AND BODY-WALL TO THE SPINAL SEGMENTS.

In noting the main features of development in early human embryos, the most convenient landmarks for describing relative positions of structures are the myotomes. By the end of the second week of embryonic development about fourteen myotomes have been distinctly differentiated in the human embryo (see Fig. 1). The formation of myotomes continues until by the end of the fourth week about thirty-eight have been differentiated (see Embryo II, Fig. 6). Anterior to the eight cervical myotomes, three occipital myotomes seem usually to be developed; posterior to the cervical region, twelve thoracic, five lumbar, five sacral, and five to six coccygeal myotomes are formed. The division of the myotomes into occipital, cervical, thoracic, lumbar, sacral and coccygeal groups depends upon the nerves and skeletal structures related to the body segments in which the myotomes lie.

The occipital myotomes are transient structures developed in conjunction with roots of the Twelfth Cranial Nerve. Before the spinal nerves have appeared they cannot with certainty be distinguished. No account of occipital myotomes is given in the description of the embryos pictured in the His Atlas. In the Mall embryos, LXXVI, CXLVIII

To the His embryo A, Fig. 7, correspond:
His's embryo Pr, length 10 mm., Atlas, Taf. XIII, Fig. 4, and Taf. X, Fig. 10.
His's embryo B, length 7 mm., Atlas, Taf. I, Fig. 1.
To No. CLXIII, Fig. 8, correspond:
Kollmann's 10.2 mm. embryo, Arch. of Anat. and Phys., 1891, Taf. III, Fig. 1.
Ruge's 9.1 mm. embryo, His's Atlas, Taf. XIII, Fig. 5, and Taf. X, Fig. 12.
To the His embryo Br, Fig. 9, corresponds:
Keibel's embryo, H. S. Bul., length 11.5 mm., Arch. für Anat. und Phys., 1891, Taf. XIX, Fig. 13a.
To No. CIX, Fig. 10, corresponds:
His's embryo S, length 12.5 mm., Atlas, Taf. XIII, Fig. 7, and Taf. X, Fig. 16.
To No. CLXXV, Fig. 11, corresponds:
His's embryo Sch_2, length 13.8 mm., Atlas, Taf. XIV, Fig. 3, and Taf. X, Fig. 18.
To No. CVI, Fig. 12, correspond:
His's embryo Br_2, length 13.3 mm., Atlas, Taf. XIV, Fig. 1.
Ruge's 13.6 mm., embryo, His Atlas, Taf. XIV, Fig. 4, and Taf. X, Fig. 19.
To No. CLXVII, Fig. 13, corresponds:
His's embryo Pr, length 14.5 mm., Atlas, Taf. XIV, Fig. 5, and Taf. X, Fig. 20.
To No. XLIII, Fig. 14 corresponds:
His's embyro S_2, length 15.5 mm., Atlas, Taf. X, Fig 21.
To No. XXII, Fig. 15 correspond:
His's embryo XCI, length 16 mm., Atlas, Taf. X, Fig. 22.
His's embryo Lt_2, length 17.5 mm., Atlas, Taf. X, Fig. 23.
His's embryo Z. W., Atlas, Taf. X, Fig. 24.

and II, the occipital myotomes have been determined by a careful study of serial sections.

The cervical myotomes are those developed in conjunction with the cervical spinal nerves. In the vast majority of instances these are eight in number. The occasional absence in the adult of a cervical vertebra indicates that in the embryo less than the normal number of cervical spinal segments sometimes develop. In all of the embryos we have studied, the arm-bud begins its development opposite the fifth to the eighth cervical segments and opposite the following spinal segment. The most caudal myotome lying opposite the arm-bud may, therefore, be taken to represent the first thoracic segment.

Between the base of the arm-bud and that of the leg-bud, as a rule, eleven myotomes intervene. A marked exception to this is found in Embryo CXLVIII, where but nine myotomes seem to lie in this region. In other instances, twelve myotomes have been pictured. Such is apparently the case in the His embryo B (Atlas, Taf. I, Fig. 1) and in the His embryo Pr (Atlas, Taf. XIII, Fig. 4). It is difficult to determine this with certainty from the figures. Variation in the number of myotomes intervening between the regions of the arm and leg corresponds with well-known variations in the length of the spinal axis in the adult.[8]

[8] Bardeen, Costo-vertebral Variation in Man, Anat. Anz., 1900.

In the His wax-models of young human embryos, Embryo Lr (No. 6) shows nine myotomes between the regions of the swellings which indicate the arm- and leg-buds. Embryo A shows fifteen myotomes between the arm and leg areas. The number of the myotomes in the thoraco-abdominal region in each of the models is probably incorrect. Fig. 5, Plate XX, of the His Atlas seems to show the usual number of thoraco-abdominal myotomes in Lr. Fig. 2, Plate I*, shows twelve instead of fifteen thoraco-abdominal myotomes in A. The model of A presents, in case of the thoraco-abdominal myotomes, conditions characteristic of the pig. Out of twelve young pig embryos of various sizes, in eleven instances we have found sixteen myotomes intervening on each side between the arm and leg regions, and in one instance fifteen. In the pig, therefore, five more body segments than in man lie between the arm and leg areas. This is also to be seen in the adult. In the pig there are two more thoracic segments than in man, and, as indicated by the nerve distribution to the abdominal wall, three more abdominal segments. The third lumbar nerve in the pig corresponds in distribution somewhat to the first lumbar in man, but it has a more extensive abdominal distribution. While therefore in man the first lumbar segment is counted as belonging to the leg area, in the pig the third lumbar segment may be considered to belong to the thoraco-abdominal region. There are six lumbar vertebræ in the pig. The furcal nerves are the fifth and sixth lumbar usually, but sometimes the fifth, probably sometimes also the sixth, may be the sole furcal nerve.

In the Keibel "Normentafeln" of the pig the number of myotomes pictured between the arm and leg areas varies from fifteen to eighteen in different embryos. It

The leg-bud arises opposite six myotomes. These usually are the twenty-fourth to the twenty-ninth, corresponding to the five lumbar and 1st sacral myotomes. Caudal to this region arise the remaining sacral and the coccygeal myotomes.

The myotomes give rise to the dorsal musculature, to the thoraco-abdominal musculature, and to the musculature of the neck, tongue (?) and caudal region. When differentiation of body musculature takes place the distinction between the myotomes becomes lost, and they can no longer be used as landmarks. This occurs by the time the embryo has reached a length of 11 mm. and an age of five weeks. Hereafter segmental skeletal structures and spinal ganglia may be used as landmarks. The former present the more stable relative conditions.

GENERAL SUMMARY OF THE MAIN EXTERNAL FEATURES NOTED IN THE EARLY DEVELOPMENT OF THE SPINAL AXIS, BODY-WALL AND THE LIMBS.

Development of the spinal axis begins in the cervical region. At the end of the second week fourteen myotomes are present (3o, 8c, 3t); at the end of the fourth week, thirty-eight (3o, 8c, 12t, 5l, 5s and 5c). During the fifth week the myotomes, owing to fusion of their dorsal surfaces, cease to be externally visible. The spinal ganglia, however, give rise to a segmentation externally visible for a somewhat longer period.

The limbs and body-wall arise in the region where the amnion joins the axis of the embryo. By the end of the second week, at the stage represented in Embryo XII, the amnion is attached directly to the axis along a line extending from a region anterior to the heart to the caudal extremity (Fig. 1). The amniotic cavity rapidly enlarges. That part of the amnion near the axis of the embryo is carried ventrally so that it closes in the viscera which have previously protruded free into the general cœlomic cavity. Finally the amnion reaches the allantoic stalk (or umbilical cord) and becomes attached to this. That part of the amnion extending from the umbilical cord to the axis of the embryo is now known as the *membrana reuniens*. It forms the chief covering of the pericardial, pleural and peritoneal cavities. Fig. 2 probably, and Fig. 3 certainly, represent stages in which the viscera are completely inclosed by the *membrana reuniens*.

is possible that no great care was taken in determining accurately the number of myotomes in this region. In the "Normentafeln" of the chick the number of myotomes pictured in this area varies from seven to ten. Apparently seven in the normal number.

During the second half of the third week the *membrana reuniens* becomes markedly thickened along its line of attachment between, usually, the fourth and twenty-sixth spinal segments. This thickening is known as the Wolffian ridge. In Figs. 2-7 the ventral margin of this ridge is emphasized by a heavy line. Opposite the fifth to the ninth and the twenty-first to the twenty-sixth spinal myotomes this thickening is especially marked. The two latter areas represent the inception of the limb-buds; the intervening area represents the rudiment of the lateral body-wall. These three areas first become well marked toward the end of the third week (Figs. 2-3).

The limb-buds increase very rapidly in size. At first the limb-buds extend directly laterally from the Wolffian ridge (Figs. 2 and 3), but as development proceeds they project more and more in a caudal direction (Figs. 4, 5 and 6). Meanwhile two distinct divisions appear in each limb-bud. The part which arises directly from the Wolffian ridge we may call the basal division. This portion continues to grow in the ventro-lateral direction taken by the limb-bud originally. Beyond this basal part the limb-bud bends in a ventro-median direction. The part of the limb-bud beyond the bend we may call the "distal part" of the limb-bud. For some time the basal portion of the limb-bud continues to grow in a ventro-lateral direction, while the distal part grows in a ventro-median direction. The limb as a whole meanwhile points distinctly in a caudal direction.

Dorsally the base of the limb becomes continuous with the dorsal margin of the Wolffian ridge, ventrally with its ventral margin. The dorso-lateral surface of the base of the limb-bud is therefore extensive; the ventro-median surface is small in area. Owing to the caudal direction assumed by the limb, the anterior (cephalic) surface of the base is extensive, while the posterior (caudal) surface is limited in extent.

The distal part of each limb-bud is flattened so that it presents median and lateral surfaces and anterior (cephalic), ventral and posterior (caudal) margins. A constriction may be seen immediately beyond the region where the distal flattened portion of the limb joins the thicker rounded base. In Fig. 8 the constriction is emphasized by heavy lines. In the arm it is seen as one looks at the limb directly from the side. In the leg, which is here slightly twisted, one may see the constriction as it appears when the limb is viewed on the anterior margin. The constriction is just beginning to appear in the leg-bud shown in Fig. B, Plate II, and in Fig. C, Plate III.

From the basal portion of each limb-bud are developed the limb-girdle (shoulder and pelvic-girdles), and the upper limb (upper arm and

thigh), from the distal portion are developed forelimb (forearm and leg) and extremity (hand and foot). The place where the distal part of the limb joins the base represents the future elbow or knee. These two joints are formed in a flexed position (see especially Figs. F, G, H and I, Plates VI-IX).

The constriction mentioned above serves to designate the differentiation of the distal part of the limb-bud into the forelimb and extremity. Opposite the constriction the forelimb is formed; immediately distal to it the extremity (Figs. 8, 9, 10, 11, 12, 13, 14, 15).

The hand and foot are at first flattened, disk-shaped bodies. Digitation is first marked on the lateral surface (see the hand in Fig. 9), and then in the free margin also (Fig. 10).

Differentiation of the base of the limb-bud is somewhat less easy to follow than that of the distal part. This is due mainly to the fact that many distinguishing structures are at first deep-seated. As the musculature, however, becomes distinctly differentiated, the various main groups of muscles give rise to distinctive external characteristics.

The limbs at the stage shown in Fig. 15, p. 9, and in Figs. F, G, H and I, Plates VI-IX, exhibit rudiments of most of the structures characteristic of the adult limbs. Much growth and shifting of parts, however, is necessary before adult conditions are reached.

The great curvature of the axis of the body opposite the limbs during their formation has been noticed by His.[9]

The arm-bud at first lies opposite the 5th to 8th cervical and 1st thoracic myotomes. As it grows in size the area of attachment to the body enlarges and extends on its cephalic side to the level of the 3rd cervical neural process (see Embryo CIX, Fig. 10). We find also that the shoulder comes to lie closer and closer to the precervical sinus until the embryo reaches a length of about 20 mm. As development still further proceeds the shoulder gradually migrates caudally and the distance between the shoulder and precervical sinus increases until the adult position is attained. Simultaneous with this caudal migration of the arm, the lower portion of the ventral wall of the neck appears.

The posterior limb arises slightly later than the anterior. Throughout its development the leg lingers somewhat behind the arm. The base of the leg-bud is at first opposite the 1st to 5th lumbar and the 1st sacral myotomes. Gradually the base extends so as to include the region opposite the second and third sacral myotomes and the upper

[9] Zur Geschichte des Gehirns etc., Bd. XIV, der Abhandl. der Mathematisch-physichen Classe der Königl. Sächs. Gesellschaft der Wissenschaften, p. 381.

extremity of the base ceases to extend over the region of the first lumbar segment. As adult condition is approached, the leg assumes a much more caudal position.

During the growth of the limbs the body-wall grows forwards at the expense of the *membrana reuniens*. In the various figures the ventral line of the body-wall is indicated. The abdominal wall has closed in against the umbilical cord by the time the embryo has reached a length of 6 cm. and an age of about 3 months.

Having thus considered the more general external features presented in the early development of the limbs and body-wall, let us turn to a consideration of the formation of the main structures within these areas.

II. Internal Structural Differentiation.

Second Week.

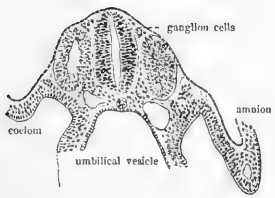


Fig. 16. Cross section through the fourth cervical segment of Embryo XII. x 55 d.

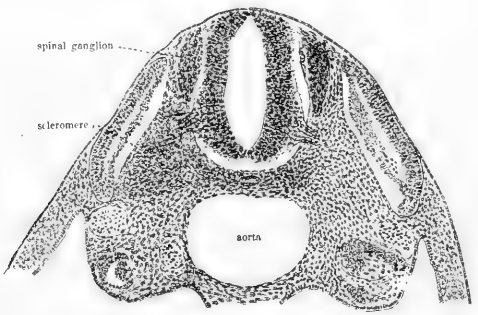


Fig. 17. Cross section through the fifth thoracic segment of embryo LXXVI. x 55 d. The right side of the section passes through the middle, the left side through the posterior third of the segment.

In an embryo of two weeks, No. XII, Fig. 1, the viscera are attached to the ventral surface of the spinal axis of the embryo, and the amnion is attached to its lateral margin. Fig. 16 shows the conditions which are seen in a transverse section through the fourth cervical segment. The spinal cord is, in this region, a closed tube, the lateral walls of which are composed of five or six layers of epithelial cells. On each side lie the myotomes. These are oval in cross-section, square with rounded corners when viewed from the lateral surface. Each has a well-marked myocœl, surrounded on all sides by four or five layers of epithelial cells. On the left in Fig. 16 the section passes through the centre, at the right it passes through the anterior margin of a myotome. Ventral to the

spinal-cord lies the notochord; on each side of this a dorsal aorta. Between notochord, aortæ, spinal-cord and myotomes lies a certain amount of mesenchyme, which arises apparently from the ventro-median surface of the myotomes.

In the region between the dorsal margin of the myotomes, the spinal-cord and the ectoderm lie spinal ganglia cells.

Ventral to the aorta on each side the splanchnopleure is attached to the axis of the embryo; ventral to the myotomes on each side, the somatopleure. The latter is continuous with the amnion. The cœlom is surrounded by epithelium, and this is surmounted by several layers of mesenchyme cells which apparently have arisen from the epithelium lining the cœlom.

Third Week.

During the third week of embryonic development marked changes take place in the spinal axis of the embryo and in the adjoining somatopleure. A section through the fifth thoracic segment of an embryo of the third week, No. LXXVI, is shown in Fig. 17. The spinal ganglia are definite, well-developed groups of cells on each side of the spinal-cord. In the spinal-cord the ventral-root zone is well marked, and the first ventral-root fibres have appeared. The myotomes have become flattened and elongated. The median surface of each of these has become converted into muscle cells, shown in cross-section in the figure. There is a single dorsal aorta, on each side of which a Wolffian body has developed. Dorsal to the Wolffian body lies the cardinal vein. The axial mesenchyme, which arises, at first at least, apparently from the myotomes, and the mesenchyme which springs from the cœlomic wall have increased very greatly in amount, and have fused so as to form a common mass of tissue which surrounds the other structures. None, however, intervenes between the dorso-lateral surface of the myotomes and the ectoderm.

A vascular plexus is developing in the mesenchyme. The axial mesenchyme of the caudal third of each segment has become dense. This is shown at the left in Fig. 17. It represents the anlage of the intervertebral disc and of the vertebral arch and costal processes. The somatopleure is considerably thicker than at the stage shown in Fig. 16. This increased thickness is due to an increase in the amount of the mesenchyme. This mesenchyme extends for a short distance dorsally between the ventral tip of the myotome and the ectoderm. The myotomes have not yet entered the body-wall.

In the region of the posterior limb the increase of mesenchyme between cœlom and ectoderm precedes the formation of muscle on the

median layer of the myotomes, the formation of the spinal ganglia, and the appearance of the ventral nerve roots. This is shown in Fig. 18, taken through the leg region of Embryo LXXVI.

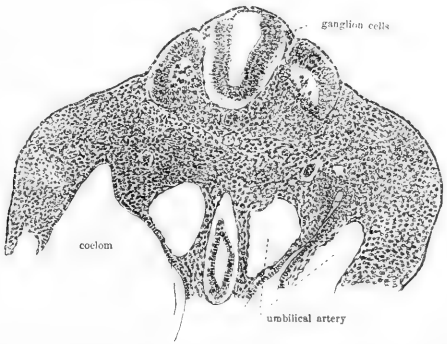


Fig. 18. Cross section through the lumbar region of Embryo LXXVI. x 55 d.

Fig. 19, taken through the leg region of Embryo CXLVIII, shows an older stage in which the limb-bud is considerably more advanced in development. The spinal ganglia are distinct. Formation of muscle cells has begun on the median surface of the myotomes.

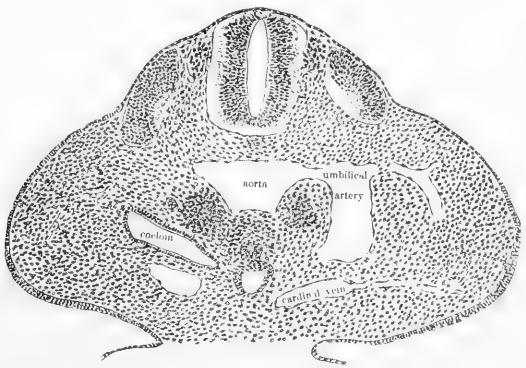


Fig. 19. Cross section through the lumbar region of Embryo CXLVIII. x 55 d.

The Wolffian ridge and limb-buds, which appear during the third week, as shown in Figs. 2, 3 and 4, consist, therefore, at the end of this period, merely of a mass of mesenchyme which intervenes between the cœlom and the ectoderm lateral to the axis of the body. This mesenchyme contains a vascular plexus. Along the free edge of the limb-buds the ectoderm consists of several layers of epithelial cells (Fig. 19).

Fourth Week.

During the fourth week certain structures extend from the spinal axis into the Wolffian ridge and the limb-buds. Let us consider first the relation of these axial structures to the body-wall and then their relation to the limb-buds.

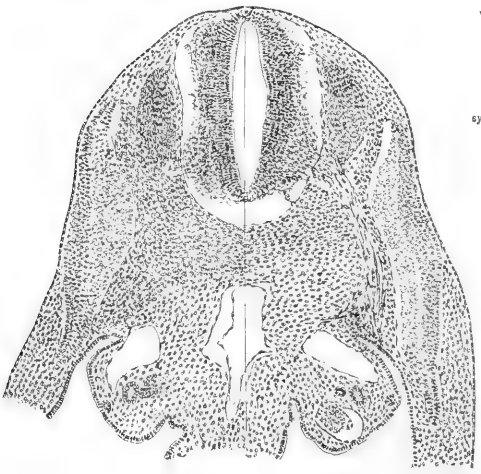


Fig. 20. Cross section through the fifth thoracic segment of Embryo II. x 55 d. The right half of the section passes through the middle, the left half through the posterior third of the segment.

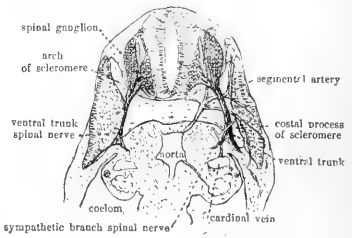


Fig. 21. Diagrammatic cross section through the mid-thoracic region of Embryo II. About 25 d. In looking at the section the spectator is supposed to be facing towards the head of the embryo. In the background one sees the fourth thoracic scleromere with arch and costal process, and at the right the intersegmental artery and the distal edge of the fourth myotome. In the foreground the spinal cord and spinal ganglia in section, and the spinal nerves of the fifth thoracic segment are shown. At the left the fifth thoracic myotome is shown in section.

Fig. 20 shows the general appearance of a typical thoracic segment (the fifth) in an embryo at the end of the fourth week. In Fig. 21 similar structures are shown somewhat more diagrammatically. Fig. A, Plate II, shows a reconstruction of the axis of the body and a part of the right lateral wall and the leg in the same embryo.

The skeletal portion of each axial segment consists of a condensation of the mesenchyme at the caudal third of the segment as shown at the left in Fig. 20. To this skeletal tissue the term scleromere may be applied. It represents the intervertebral disc, the arch or neural process and the costal process of a segment of the future spinal column. The general form of the scleromeres at this stage may be readily seen in Fig. A. The scleromeres do not as yet extend into the body-wall.

The general form of the myotomes may be seen in Fig. A. At the

right the 11th and 12th thoracic and the 1st and 2d lumbar myotomes are viewed from the side and slightly in front. At the left a portion of the median surface of several myotomes may be seen. In Fig. 20 two myotomes are shown in cross-section. The myocœl has disappeared. The median surface of each myotome has been entirely converted into musculature. The lateral surface and ventral and dorsal tips are covered by epithelium. A certain portion of the dorsal surface of the thoracic myotomes is, however, converted into musculature. This is shown in the myotomes at the right in Fig. A. The thoracic myotomes extend for a short distance into the body-wall. In Fig. 20 this is shown in a cross-section. In Fig. A the projecting tips of the myotomes may be seen through the membrane lining the cœlom.

The thoracic spinal nerves project for a shorter distance into the body-wall than do the myotomes. Each is divided at the dorsal margin of the cœlom into two portions, a median which becomes the sympathetic ramus, and a lateral which extends outwards between the median surface of the myotome and the lining membrane of the cœlom and becomes the ventral trunk of the spinal nerve (see Figs. A, 20 and 21).

From the aorta intersegmental arteries arise and send branches dorsally toward the spinal ganglia, laterally between the myotomes and ventrally into the body-wall (see Fig. 21). A considerable vascular plexus is developed in the mesenchyme. The general relations of the latter are shown in Fig. 20. The venous blood is collected in the cardinal veins and in branches of the umbilical vein.

The general relation of the formed structures of the axis of the body to the leg-bud at the end of the fourth week are shown in Fig. A, Plate II. The limb-bud lies opposite the five lumbar and the first sacral segments. The cœlom extends to a point opposite the first sacral segment, but in the region of the limb it does not extend as far dorsally as in the thoracic region. From the model represented in Fig. A, several of the myotomes of the left side, the axial mesenchyme, the aorta, the left cardinal vein, the intestines and urino-genital organs have been removed. A portion of the right cardinal vein and a portion of the right umbilical artery are shown, 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 sinusoidal 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 do not extend into the limb-bud.

By following the successive spinal segments from the fourth sacral to the first lumbar in Fig. A, an idea may be gained of the development of segmental structures in the axial region. The scleromeres are represented at the caudal third of each segment. On the left side of the body the myotomes are omitted from the 3d sacral to the 3d lumbar segments. The spinal nerves first appear in the first and second sacral segments.

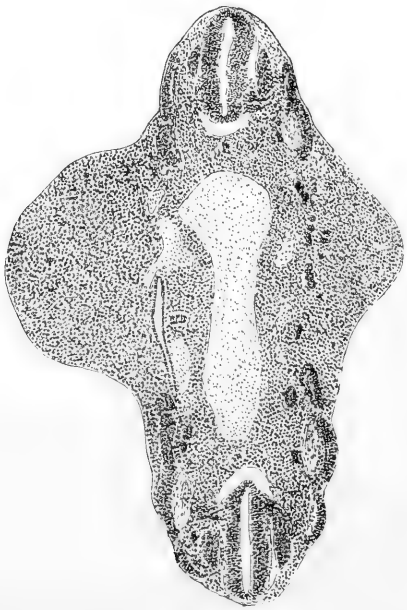


Fig. 22. Tangential section through the leg region of Embryo II. 25 d.

Fig. 22 shows the general conditions existing in the limb region of Embryo II when seen in section. At the left the leg-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 the arm region of Embryo II, the cervico-brachial plexus is formed and limb nerves extend into the limb-bud. The conditions are essentially similar to the conditions found in the leg region of Embryo CLXIII and described below.

Fifth Week.

During the fifth week of development a considerable amount of organization occurs in the spinal axis, the body-wall and the limbs. The nature of the processes taking place are indicated in Embryo CLXIII (length, 9 mm.; probable age, $4\frac{1}{2}$ weeks).

The structure of the back, the limbs and body-wall in this embryo is shown in Fig. B, Plate II, and Fig. C, Plate III. The areas mentioned are drawn from reconstructions. The remaining parts of Fig. C are drawn from an excellent photograph.

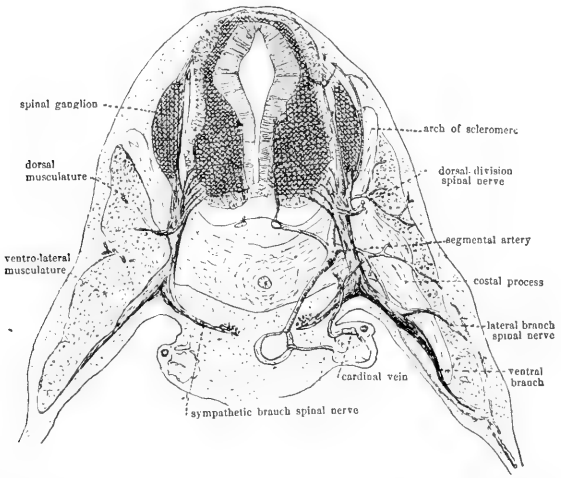


Fig. 23. Diagrammatic cross section through the 5th-6th thoracic segments of Embryo CLXIII. x 25 d. The general arrangement of the structures represented is like that described for Fig. 21.

Fig. 23 shows diagrammatically the general nature of the structures in a typical thoracic segment (the 6th) of Embryo CLXIII. The changes taking place in the thoracic region during the first half of the 5th week may be readily followed by comparing Fig. A with Fig. B, and Fig. 21 with Fig. 23.

The skeletal portion of the segment consists in Embryo CLXIII, as in Embryo II, of a condensed mesenchyme at the distal third of the segment, but the scleromere is far more definitely outlined. Neural and costal processes are well developed. The latter present something of the general form of ribs in the thoracic region (see Fig. 23 and Fig. B). A sheet of condensed mesenchyme connects the neural and costal process of the scleromeres of neighboring segments. That connecting the

neural processes is shown in Fig. B at the right. That portion of each scleromere representing an intervertebral disc is likewise united ventrally and dorsally with its neighbors by a dense sheet of tissue at the periphery of the disc. The ventral portions of these sheets of tissue are represented in Fig. B. Within the space lying between the scleromeres is developed the chondrogenous tissue which gives rise to the vertebral bodies. Marked alterations have taken place in the myotomes. In the thoracic region the two layers of the myotome have, with the exception of the dorsal and ventral tips in the more distal segments, become converted into musculature (see Fig. 23 and Fig. B). As shown at the left in Fig. 23, the myogenous tissue which has arisen from the myotomes is being divided into three portions—a dorsal, a lateral and a ventral—by the ingrowth of a vascular mesenchyme. The finer changes taking place during this period are essentially similar to those previously described as taking place in the body-wall of the pig.[10]

The tissue of the superficial lateral layers of the myotomes has formed into a continuous layer (see Fig. C, Plate III). Segmentation, however, is still visible.

Both the costal processes of the scleromeres and the myotomes have extended well into the body-wall (Fig. 23).

The thoracic spinal nerves likewise have kept pace in growth with the myotomes. The ventral extremities of the spinal nerves are caught between the tips of the myotomes (see Fig. B, Plate II). From the spinal nerves, dorsal and lateral branches have arisen as well as the sympathetic. A sympathetic cord has arisen from the extremities of the sympathetic rami.

The segmental arteries (see Fig. 23) are similar in nature to those of the stage shown in Fig. 21, but the branching is more extensive. The mesenchyme is much more developed and now surrounds all formed structures in each spinal segment. It has begun to invade the myotomes.

In the region of the posterior limb bundles of 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 (Fig. B). Within the leg-bud 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 mass is made diagrammatically sharp. The femoral portion of the

[10] Bardeen, 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. Vol. IX, Johns Hopkins Hospital Reports, 1900.

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 of the plexus into the four main divisions which form the origin of the four chief nerves of the limb. The main artery and vein of the limb are represented, but smaller in proportion to the other structures than in nature. The border vein at this period is well developed. The conditions just described are well shown in Fig. C, Plate III, which represents the conditions seen in the limb from the lateral side after removing the ectoderm and the undifferentiated mesenchyme. It is to be noted that the myotomes do not extend into the limb-bud.

The arm is somewhat more advanced in development than the leg. The following description applies to the conditions represented in Fig. C, Plate III. A detailed account of the structure of the arm at this stage is reserved for a later paper.

Lateral to the myotome system in the arm region is an ill-defined mass of mesenchyme extending from the upper cervical to the 7th thoracic myotomes. At the level of the 1st and 2d ribs it divides into several masses. The first passes ventral to the arm and brachial plexus. The main portion of it joins the arm pre-muscle sheath. From this mass the pectoral muscles develop, hence we may designate it the pectoral pre-muscle mass. It is continuous ventrally with an irregular mass of condensed tissue, the ventral neck pre-muscle mass. The second division of the lateral pre-muscle mass passes dorsal to the arm and brachial plexus and joins the arm pre-muscle sheath. It constitutes the latissimus dorsi pre-muscle mass. The third division, parallel to the ventral portion of the cervical myotome column, represents the levator scapulæ and serratus anterior pre-muscle mass. Lateral to it is an ill-defined mass of pre-muscle tissue. The fourth and most dorsal division is thinner and less clearly marked than the others. We may call it the rhomboid pre-muscle mass. The caudal limit of the trapezius pre-muscle mass appears at the upper end of the arm region. It is at the level of the 4th cervical neural processes, and from here the muscle mass extends to the occipital region. Most of the arm pre-muscle sheath which surrounds the skeletal core has been dissected away. Toward the distal end of the arm the skeletal and pre-muscle tissues blend (Fig. C). Beyond this point differentiation is less advanced. The proximal portion of the arm sheath is continuous with that around the scapula.

Part of the skeletal core is seen projecting caudally beyond the cut edge of the pre-muscle sheath. The lower end of the humerus, ulna

and radius are represented. The ulna and radius are continuous with the hand plate, which is composed of less differentiated tissue. There is a slight bend at the elbow. The upper end of the humerus is continuous with the scapula. The scapula is a flattened oval mass embedded in the scapular pre-muscle tissue. No coracoid or arromiom processes are present. The clavicula is not present. The skeletal core is composed of dense mesenchyme. It shades off, however, into the surrounding pre-muscle sheath.

The brachial plexus is formed by the 5th to 8th cervical and 1st thoracic nerves. The spinal nerves, as well as the plexus they form, have scarcely any caudal inclination, but pass ventrolaterally into the arm. The plexus is fairly well formed. The main nerves arising from it are present. The presence of the condensed skeletal core separates these into two groups. The musculo-spiral and circumflex on the dorsal and the ulnar, median and musculo-cutaneous on the ventral side. In Fig. C only the tips of the musculo-spiral and median nerves are shown. At this stage they have scarcely grown to the elbow.

Sixth Week.

During the sixth week the limbs and the body-wall and the back begin to approach the structural conditions characteristic of the adult.

The conditions present at the end of the fifth week are shown in Embryo CIX; length, 11 mm.; age, about 5 weeks. This embryo is pictured in Figs. D and E, Plates IV and V. These figures, with the exception of the head in Fig. D, are drawn from wax-plate reconstructions. Fig. 24 represents a cross-section through the 6th to 7th thoracic segment of a slightly older embryo, CVI (length, 15.5 mm.; age, about 5½ weeks).

The typical thoracic segment during the first half of the sixth week exhibits the following conditions:

The skeletal structures have begun to assume adult form. Between the intervertebral discs the bodies of the vertebræ are now formed of chondrogenous tissue. This may be seen in the dotted area of the scleromere in Fig. 24 and in the darker areas of the spinal column in Fig. E. The chondrogenous tissue extends into the neural and transverse processes of the scleromere (see the left side of Fig. 24). Each costal process extends considerably further ventrally than at the stage shown in Fig. 23. Within the dense tissue of the costal process chondrogenous tissue is formed similar to, but without direct connection with, that of the vertebral body and transverse process. A lateral view of the

neural and transverse processes of the vertebræ is shown in the cervical region in Fig. D. The extent of development of the ribs is shown in Fig. E.

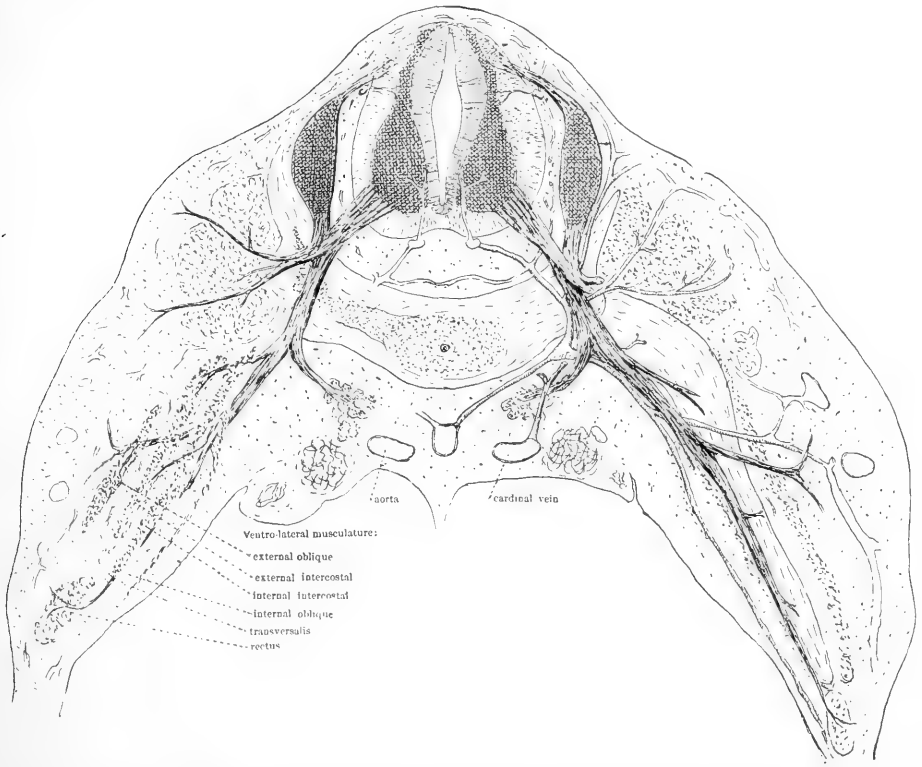


Fig. 24. Diagrammatic cross section through the 6th-7th thoracic segments of Embryo CVI. x 25 d. This figure is made to correspond with Figs. 21 and 23. See legend for Fig. 21.

The musculature has undergone most marked changes. The dorsal musculature is slightly separated from the ventro-lateral by vascular mesenchyme. The dorsal musculature is further subdivided into three muscle columns. These correspond to the ileo-costal, longissimus dorsi and spinalis groups of dorsal muscles in the adult. The ventral musculature is likewise subdivided into two main portions, the rectus muscle and the lateral musculature. The last is further subdivided into external oblique, the intercostals, the internal oblique, and the transversalis muscles. In the intercostal muscles alone is the segmentation characteristic of the myotomes fully maintained.

In Fig. D the separation of dorsal from ventro-lateral musculature is clearly shown. The dorsal musculature is shown divided into three columns in the upper thoracic region; caudally differentiation is not so extensive. The musculature of the external oblique may be seen, in Fig. D, covering the external intercostal muscle, the ribs and in part the rectus musculature. In Fig. E the rectus musculature, internal intercostal and internal oblique may be seen. Differentiation, however, is not so far advanced at the stage shown in Figs. D and E as it is at the stage shown in Fig. 24. The ventro-lateral abdominal musculature of Embryo CIX is connected by an irregular dense band of tissue with the pubic process of the anlage of the pelvic bone. This band of tissue is represented with diagrammatic distinctness in the drawings.

The neural apparatus has undergone rapid development. Fig. 24 shows clearly the main branches arising from the typal thoracic nerve. Muscle twigs are arising. The general appearance of the spinal nerves at this stage is shown in Fig. E.

The mesenchyme is extensive in amount. The blood-vessels are similar in general distribution to those described in Embryo CLXIII, but the vascular plexus is more extensively developed.

In the posterior limb the central skeletal mass has assumed somewhat definite outlines. Fairly good views of it are presented in Figs. D and E. The pelvic portion of the skeleton of the limb 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. It is indistinctly shown in Fig. D. The tibia and fibula are of fairly definite form (Figs. D and E). The skeleton of the foot has the form shown in Figs. D and E. It is composed of condensed mesenchyme. No accurate division into parts can be distinguished.

The main nerve trunks have continued their growth into the limb. From them many of the principal muscular and cutaneous branches have sprung. The general distribution of the lateral nerves of the limb, the femoral and peroneal, and their branches, may be seen in Fig. D. That of the median nerves of the limb, the obturator and tibial, in Fig. E. In both figures the anterior border nerves (the ilio-hypogastric and genito-crural) and the posterior border nerves (the pudic and posterior-cutaneous) are shown.

About the main branches of the nerves of the limb a differentiation of musculature has begun. This is indicated in Figs. D and E. In Fig. 25 are shown the appearances in cross-section presented by the early developing musculature of the leg. The blood-vessels of the limb are shown in Figs. D and E. The sciatic artery is still the chief source of

supply, but the femoral and obturator arteries also have appeared. The blood is carried into the cardinal (iliac) vein partly by the femoral vein and partly by the sciatic. The formed structures of the limb are surrounded by a vascular mesenchyme.

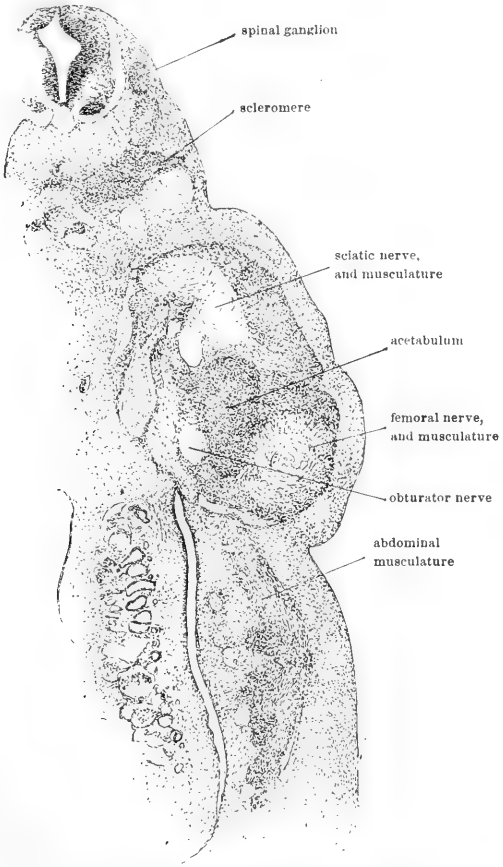


Fig. 25. Section through Embryo CIX. x 25 d.

The conditions existing in the arm are considerably in advance of those in the leg. The following description of the conditions in the arm region can be followed from Figs. D and E, Plates IV and V.

The lateral pre-muscle mass has become completely divided into several groups. The tissue of these groups is now fibrillated. The first division, the one which passes ventral to the brachial plexus, is seen in Plate V. It constitutes the pectoral muscle mass, representing

both pectoralis major and minor muscles. This mass extends from the level of the third rib to the humerus and clavicle. There is no attachment to the ribs. The intercostal muscles have been dissected away in Plate V to show its costal end. The second division of the lateral pre-muscle mass has developed into the latissimus dorsi and teres major muscle mass. It extends from the level of the 4th rib to the humerus, where it blends with the scapulo-humeral mass. Its development and differentiation have not proceeded so far as the pectoral mass. The third division of the lateral pre-muscle mass has developed into a long muscle extending from the 1st cervical vertebra to the 9th rib. Digitations extend to the transverse processes of the cervical vertebræ and to the cephalic 9 ribs. The muscle lies in a more median plane than the scapula and has as yet no attachment to it. It represents the levator scapulæ and serratus anterior muscles. The trapezius mass has extended to a lower level than found in CLXIII. There is no scapular attachment. Only the ventral half of the mass pictured in Plate IV consists of muscle fibres, the remaining connective tissue portion connects with the dorsal ends of the neural processes.

Considerable differentiation in the pre-muscle sheath has taken place. The scapulo-humeral mass is with difficulty separated into the various muscles. These are more blended into a single mass than would appear from Plates IV and V. Here portions of the deltoid and trapezius have been dissected away.

The extensor mass of the forearm can be separated into three groups which are more or less blended however. The larger superficial mass has been partially dissected away. It represents the extensor digitorum communis, extensor carpi ulnaris, and extensor digiti quinti proprius muscles. The second group arises beneath the first group, taking a course at nearly right angles to it. It represents the deep extensor muscle of the forearm. These two groups fuse distally with the general condensed mesenchyme of the digits, where all traces between pre-muscle and pre-cartilage are lost. The third group represents the brachio-radialis and the extensor carpi radiates longus et brevis. A portion of the brachialis can be seen in Plate IV. The flexor surface of the arm is shown in Fig. E, Plate V. The biceps and coracobrachialis are obscured by the pectoral mass. The flexor mass of the forearm has split into two layers. It shows less differentiation than the extensor mass.

The arm skeleton shows considerable advance. The shape of the scapula has changed, both coracoid process and acromion are present and of large size. The clavicle has begun to develop and consists of

an ill-defined mass of condensed tissue projecting about one-half the distance from the acromion to the tips of the first rib. The humerus is fairly well defined and is continuous with the scapula as well as the ulna and radius. The elbow bend is well marked. The ulna and radius are farther advanced than in Embryo CLXIII. They end distally in the carpal plate. In the carpal plate indications of formation of the carpal elements are seen. The digits consist of undifferentiated tissue in which are blended skeletal muscle and tendinous elements. The humerus, ulna and radius have a core of hyaline cartilage. The rest of the skeletal tissue consists of condensed mesenchyme and pre-cartilage.

In Plate IV are shown the circumflex, radial and musculo-cutaneous nerves. The brachial plexus with portions of the nerves arising from it are seen in Plate V. The plexus is well formed. It has only a slight caudal inclination. The spinal accessory nerve is seen on the median surface of the trapezius. Branches from the 3d and 4th cervical nerves join it.

Seventh Week.

By the end of the seventh week most of the structures characteristic of the adult back, body-wall and limbs have appeared. Subsequent development depends in the main upon growth and upon relative shifting of parts.

The structures of the abdominal wall in a seven-week embryo (XXII, length 20 mm.) are shown in the Figs. F, G, H and I, Plates VI, VII, VIII and IX. The vertebræ are composed of embryonic hyaline cartilage. Each presents a neural and a transverse process on each side. The cartilaginous portions of the vertebræ are shown in the upper thoracic region of Fig. H. The ribs are likewise composed of embryonic cartilage, shown in the same portion of the figure. The cartilage of the ribs is at no time continuous with that of the vertebræ. The ribs and vertebræ are surrounded by a dense mesenchyme. This is continuous from the ribs to the transverse process of the vertebræ. Between the bodies of the vertebræ it forms the intervertebral discs (Fig. I). It is continuous over the surface of the spinal column. From it are developed the ligaments characteristic of the thorax and spinal column together with the perichondrium and periosteum. No thirteenth rib is present in this embryo or in any of the other young embryos we have studied. Fig. 26 shows at the right the portion of the skeleton composed of embryonic cartilage, at the left the covering of dense mesenchyme.

The musculature of the back and abdominal walls has a general resemblance to that of the adult. Fig. I shows the musculature of the abdomen and thorax as seen from within, Figs. F and G that of the more superficial layers of the abdomen and thorax, and Fig. H that of the deeper layers of the abdomen and thorax. The dorsal muscles are not clearly shown in any of the figures, but they are divisible into the three distinct groups, the ileo-costal, longissimus dorsi, and spinalis muscles, characteristic of the adult.

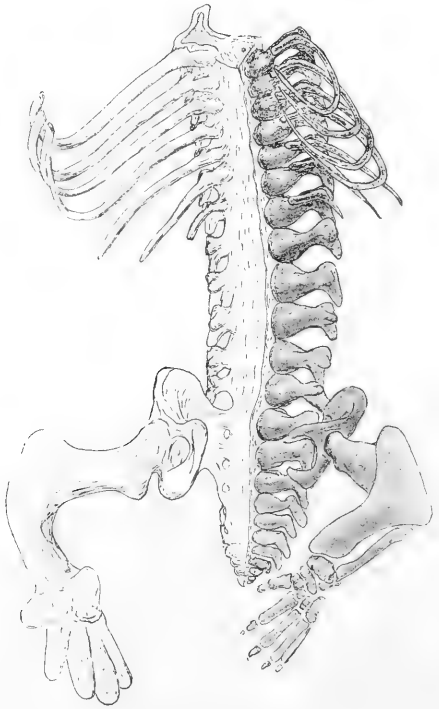


Fig. 26. Skeleton of distal half of Embryo XXII. At the left side the covering of dense embryonic connective tissue is shown, at the right the parts composed of embryonic cartilage. x 10 d.

The nerves, like the muscles, have a distribution essentially similar to that found in the adult. The figures indicate with sufficient clearness the distribution of the thoraco-abdominal and border nerves.

The main blood-vessels are those characteristic of the adult.

In the posterior limb the skeletal tissue has undergone extensive differentiation. The rudiments of all of the bones of the leg may be seen in the form of cartilage except that the terminal phalanges of the

three outer toes have not yet appeared (see Fig. 26, right side). The cartilaginous skeleton of the limb, like that of the spine, is covered by a dense mesenchyme. Torsion has not yet begun at the ankle-joint.

The musculature of the posterior limb is so far differentiated that all of the individual muscles characteristic of the adult may be distinguished except the lumbricales. The muscles lie in distinct groups, as is shown in the various figures.

The femoral or extensor group of muscles is shown in Figs. F and H. The groups of muscles belonging to the peroneal nerve and its branches, the gluteal, peroneal, and pedal extensor muscles may be seen in Figs. F and H. The adductor or obturator group of muscles is best seen in Fig. G. The groups of muscles belonging to the tibial nerve may be seen in Fig. I. A detailed account of these muscles is reserved for a subsequent article.

The nerves of the posterior limb, like the muscles, are so well developed that most of them may be readily compared with those of the adult. To reach the adult position a considerable amount of shifting, however, must take place.

At the period under consideration the blood-vessels of the limb are those characteristic of the adult. The main artery and the chief vein are the femoral.

An idea of the general condition of the tissues of the limb at this stage may be obtained from Fig. 27, which represents a photograph taken through a section passing through the limb region.

The anterior limb presents similar conditions of structure.

The muscular system shows well-marked fibrillation. All of the muscles of the adult arm are present and in about the relative adult positions.

The bones of the arm are represented by hyaline cartilage except the distal row of phalanges of the 2d to 5th digits. This row is represented by masses of undifferentiated condensed tissue, into which the long extensor and flexor tendons merge.

The clavicle is well developed and extends to the 1st rib, where it comes in contact with the sternal anlage. The sternal anlage is composed of condensed mesenchyme. The ribs, vertebræ and their neural processes are composed of cartilage. All of the cartilages of the arm are surrounded by a condensed mesenchymal sheath, the perichondrium.

The nervous system presents nearly the adult conditions. It has not been possible, however, to resolve the brachial plexus into its usual distinct cords. They appear from study of sections and the reconstruction to be fused into one mass.

SUMMARY OF THE EARLY DEVELOPMENT OF THE BACK, LIMBS AND BODY-WALL.

In an embryo of two weeks, XII, Figs. 1 and 16, fourteen myotomes are present, three occipital and eleven spinal (8c, 3t). In an embryo of

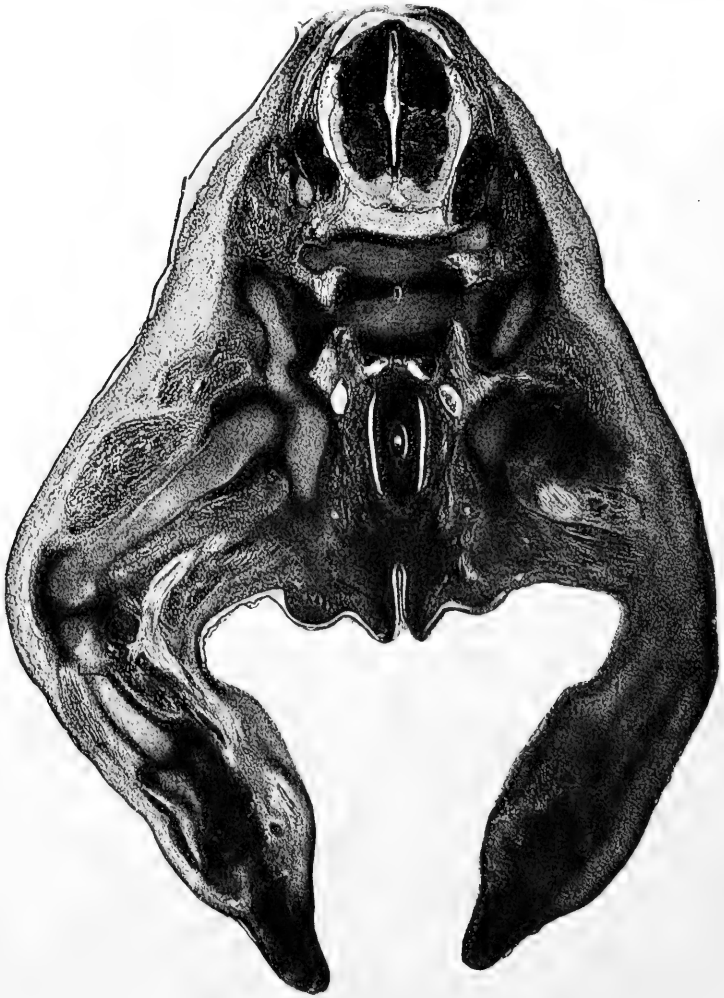


Fig. 27. Photograph of a section through the leg region of Embryo XXII.

four weeks, II, Figs. 6, 20, 21 and A, thirty-eight myotomes have been counted, three occipital and thirty-five spinal (8c, 12t, 5l, 5s and 5c). At this stage the formation of myotomes ceases. Soon hereafter the

occipital myotomes disappear. From the thoracic myotomes processes enter the body wall. During the fifth week the myotomes give rise to a dorso-ventral muscle-mass in which the segmentation characteristic of the myotomes mainly disappears. This muscle-mass becomes divided longitudinally into two great divisions—a dorsal and a ventro-lateral. Into the composition of the dorsal division all of the spinal myotomes enter. From it is derived the dorsal musculature of the adult. The ventro-lateral muscle-mass is formed from the processes which extend from the thoracic myotomes into the body wall. From it are derived the intrinsic muscles of the thorax and abdomen. The differentiation of these muscles takes place during the fifth, sixth and seventh weeks.

From the median surface of the myotomes near the ventral margin mesenchyme springs to surround the intrinsic structures of the spinal axis (Fig. 16). This mesenchyme is at first non-segmental in distribution. Gradually, however, it becomes denser at the posterior third of each spinal segment. This condensed tissue forms the scleromeres. From the scleromeres are developed the intervertebral discs, the arches and transverse processes of the vertebræ, and the ribs. Between the scleromeres the bodies of the vertebræ are formed. The vertebral column at first surrounds only the ventral half of the spinal-cord. It is at a comparatively late period that the vertebral arches from each side meet dorsally to form the vertebral spines.

Owing to the accurate studies by His, the main stages in the development of the spinal-cord and early formation of the spinal nerves are too well known to demand further description. We find, however, that the dorsal divisions of the spinal nerves are given off after the division of the spinal nerve into somatic and sympathetic branches, a period later than that described by His. When the dorsal musculature begins to be formed from the tissues derived from the myotomes, the dorsal divisions appear and pass into the differentiating musculature, where they give rise to the characteristic median and lateral trunks from which muscular and cutaneous branches spring. The ventral trunks of the spinal nerves in the cervical and lumbo-sacral regions unite to form plexuses from which in turn the nerves of the neck and limbs arise. In the thoracic region the ventral trunks pass into the body wall as intercostal nerves. Sympathetic branches are given off at the end of the fourth week, at the period when the thoracic nerves reach the dorsal margin of the cœlom. The lateral and ventral cutaneous branches are given off during the fifth, the main muscular branches during the sixth week.

Soon after the two dorsal aortæ fuse into a single dorsal aorta intersegmental arteries are given off. From these, main branches pass between the myotomes, between the spinal ganglia and to the ventral surface of the spinal-cord. Longitudinal anastomosing branches are formed between these vessels and an extensive vascular plexus arises. The blood is collected again in the cardinal veins and into the abdominal branches of the umbilical vein.

The limbs and body-wall are developed in the Wolffian ridge. This first appears as a thickening of the *membrana reuniens* along its attachment to the axis of the body between the 4th and 26th spinal segments. The limbs are developed from special bud-like projections from the Wolffian ridge, the anterior extremity appearing opposite the 5th to 9th spinal segments, the posterior extremity opposite the 21st to 26th. At the end of the third week the Wolffian ridge and limb-buds are well marked, but are without special internal differentiation.

The body-wall is developed by an ingrowth into the Wolffian ridge of processes from the myotomes, scleromeres, nerves and blood-vessels belonging to the twelve thoracic spinal segments, and a gradual differentiation of adult structures from embryonic. Ingrowth begins during the fourth week, structures essentially similar to those characteristic of the adult are differentiated by the end of the sixth week. The body-wall does not complete its growth to the midline, however, until toward the end of the third month.

Into the limb-buds blood-vessels and nerves extend from the axis of the embryo, but neither myotomes nor scleromeres send processes into the limbs. The skeletal and muscular structures of the limb are differentiated from the mesenchyme of the limb-bud. Ingrowth of blood-vessels precedes ingrowth of nerves. The brachial plexus is formed and nerves grow into the anterior limb during the latter half of the fourth week. The lumbo-sacral plexus is formed, and nerves grow into the posterior limb during the first half of the fifth week. Skeletal differentiation begins in the region of the shoulder or hip, and extends distally and proximally. This differentiation immediately precedes ingrowth of the nerves of the limbs. The skeletal structures serve in part to guide the nerves in their distribution. Muscle differentiation immediately follows the entrance of a motor nerve into a given region. From this, however, it must not be concluded that a causal connection exists between the nerves and differentiation of muscles. [11]

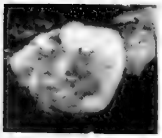
[11] See: Leonowa. Ein Fall von Anencephalie combinirt mit totaler Amyelie Neurol. Centralbl. Leipsic. Bd. XII (1893), s. 218, 263.

The structures of the upper arm and thigh are differentiated before those of the forearm and leg, and the latter before the hand and foot. Differentiation in the anterior limb precedes that in the posterior limb. Most of the main structures of the anterior limb may be distinguished at the end of the sixth week; most of those of the posterior limb at the end of the seventh week.

During the first two months of embryonic life, therefore, are developed the rudiments of the muscles, nerves, blood-vessels, and skeletal structures characteristic of the back, the body-wall and the limbs. Adult conditions are reached by an increase in size and complexity of the various organs and by a relative shifting of parts.

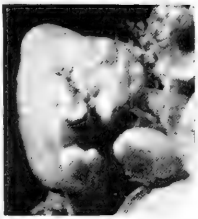
PLATE I.

A series of photographs of human embryos in the collection belonging to Dr. Mall in the Anatomical Laboratory of the Johns Hopkins University. The Roman numerals refer to the numbers by which these embryos are designated. The Arabic numbers indicate the ratio between the size of the photographic image and the size of the embryo.

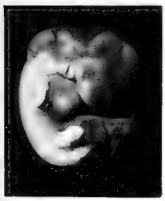


CXLVIII—3-1

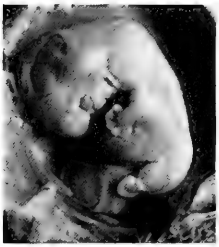
LXXX—8-5



CLXXV—23-13



CLXIII—2-1



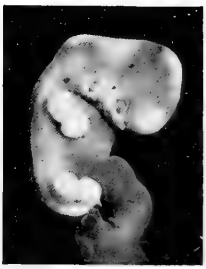
CLXVII—24-13

CLXXV—23-13

CLXIII—2-1

CIX—2-1

CIX—2-1



CVI—55-37

XXII—2-1

PLATE II.

FIGURE A. Magnification about 20 d.

Drawing from a reconstruction of the region of the posterior extremity in Embryo II; length, 7 mm.; age, 26 days. The xvii (9th thoracic) to the xxix (4th sacral) spinal segments, and, at the left, the right leg and a portion of the body-wall are represented. From the region ventral to the spinal-cord unformed mesenchyme, the aorta and left cardinal vein, and the smaller blood-vessels, the intestines and urino-genital organs have been removed. From the spinal segments the myotomes of the left side have been removed with the exception of the last two thoracic, the first two lumbar and the fourth sacral. Half encircling the spinal-cord the scleromeres may be seen at the distal third of each spinal segment. The chorda dorsalis runs ventral to the midline of the spinal-cord. The 9th to the 12th right thoracic myotomes and nerves of the right side may be seen extending for a short distance behind the lateral surface of the cœlom. The thoracic nerves give off sympathetic branches at the dorsal margin of the cœlom. The first four lumbar nerves give off spreading branches towards the limb-bud. The 5th lumbar and 1st sacral nerves are but slightly developed. The umbilical artery curves about the posterior tip of the cœlom and sends an arterial branch into the leg-bud. Below this the cardinal vein sends a branch into the limb.

FIGURE B. Magnification about 20 d.

Drawing from a reconstruction of the region of the posterior extremity in Embryo CLXIII; length, 9 mm.; age, about 4½ weeks. The xvi (8th thoracic) to the xxx (fifth sacral) spinal segments are represented. The right leg and a portion of the right body-wall are shown. From the region ventral to the spinal-cord unformed mesenchyme, the blood-vessels, the lining membrane of the cœlom, the intestine, and the urino-genital organs have been removed. The 11th and 12th thoracic myotomes only are represented on the left side. The 9th to the 12th myotomes, costal processes and spinal nerves extend ventrally in the right body-wall. Lateral and dorsal branches have arisen from the spinal nerves. The sympathetic cord receives branches from the thoracic and the first two lumbar nerves. The five lumbar and the first two sacral nerves combine to form a plexus. From this the four main nerve trunks of the posterior limb are beginning to spring. The sciatic artery and vein are represented entering the limb. At the centre of the base of the limb-bud the femur and hip-bone are beginning to be differentiated by the formation of a dense mass in the mesenchyme. The mesenchyme lying median to the limb-bud has been removed so as to expose this skeletal mass and the lumbo-sacral plexus.

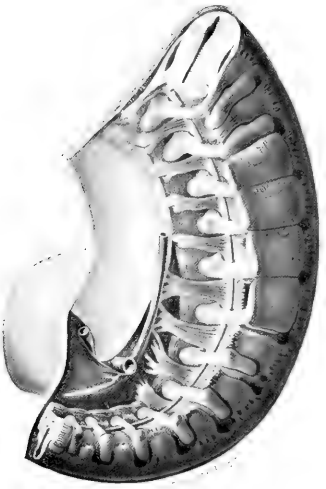


FIG. A.

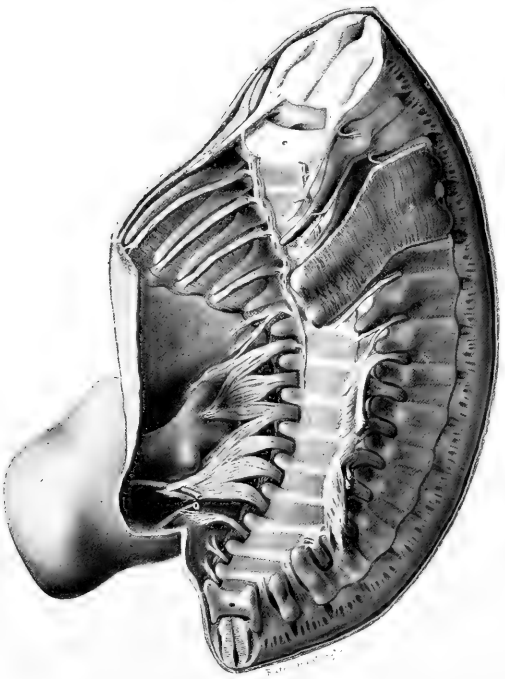


FIG. B.

PLATE III.

FIGURE C. Magnification about 15 d.

Lateral view of Embryo CLXIII; length, 9 mm.; age, 4½ weeks. The areas from which the skin has been removed are drawn from reconstructions, the remaining portions are drawn from excellent photographs. The myotomes hide from view most of the deeper structures of the back and body-wall. The superficial tissue of the myotomes has to a certain extent fused, so that segmentation is becoming indistinct. In the region of the arm, certain dense masses of tissue are represented in which later the musculature of the arm is differentiated (see p. 23). In the region of the forearm and hand this "premuscle" tissue has been removed so as to disclose the dense mass of mesenchyme which at the centre of the limb-bud represents the forerunner of the skeleton. The skeletal tissue is represented with sharper outlines than in nature. Ulna, radius and hand plate are shown. The musculo-spiral and median nerves may be seen reaching about to the elbow.

In the region of the leg the superficial tissue has been moved so as to disclose the border vein, the sciatic artery, the skeletal rudiment of the femur and hip-bone and the lumbo-sacral plexus. Into the formation of the latter enter the five lumbar nerves and the first two sacral.

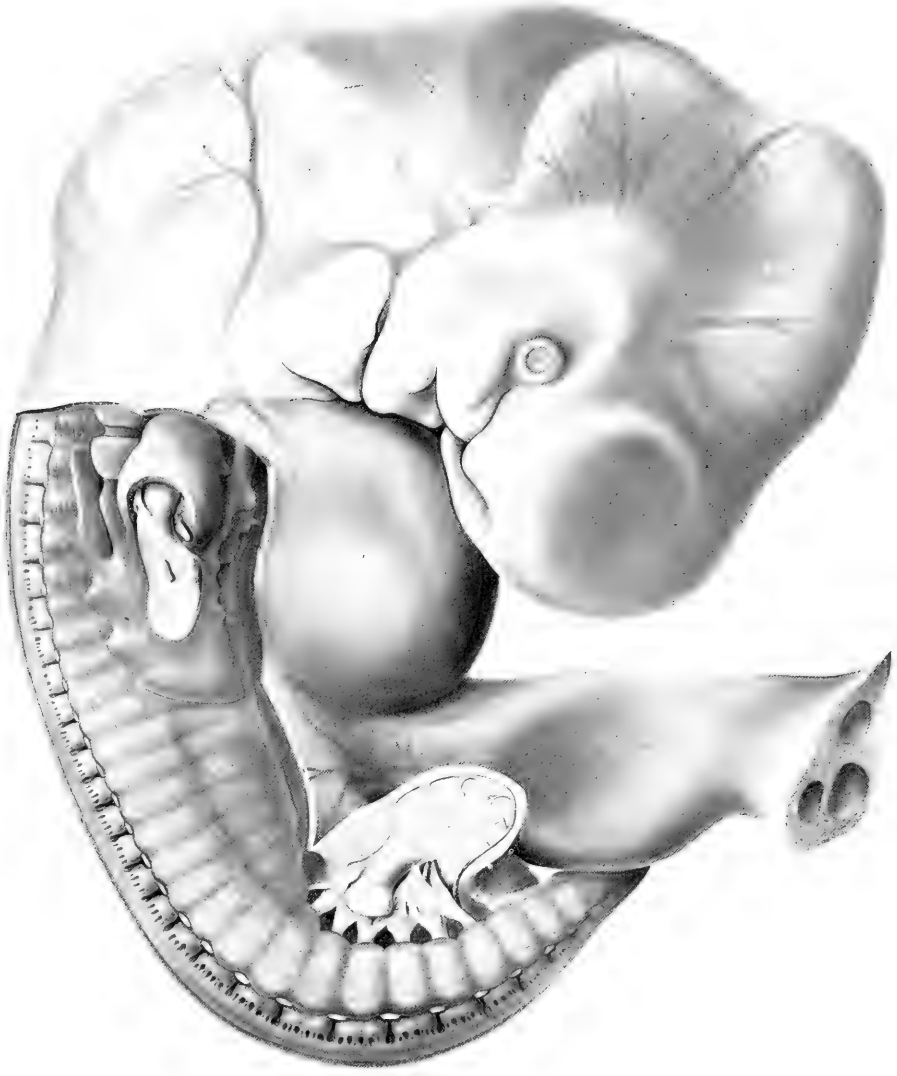


FIG C.

PLATE IV.

FIGURE D. Magnification about 12 d.

Lateral view of Embryo CIX; length, 11 mm.; age, about 5 weeks. The areas from which the skin has been removed are drawn from wax-plate reconstructions, the remaining portions are drawn partly from photographs, partly from an embryo of corresponding age. The arches and transverse processes of the 4th to 8th cervical vertebræ have been exposed by the removal of the dorsal musculature in that region. The embryonic cartilage of which these structures are formed has been exposed by the removal of the perichondrial mesenchyme. The dorsal musculature has likewise been removed from the 5th lumbar and first three sacral segments. In this region, however, is shown the dense mesenchyme which incloses the cartilaginous portions of the spinal column.

The heads of the first three ribs may be seen median to the transverse processes of the first three thoracic vertebræ. The third to the eleventh ribs may be seen through the lateral musculature of the body-wall.

The dorsal musculature is distinctly separated from the ventro-lateral. In the thoracic region little evidence remains of segmentation in the dorsal musculature. In the lumbar, sacral and coccygeal regions myomeric structure is still visible. The ventro-lateral musculature, which has developed from processes from the twelve thoracic myotomes, is beginning to assume a differentiation into the muscles characteristic of the thorax and abdomen.

The dorsal divisions of the spinal nerves are shown in the regions where the vertebræ are exposed. The lateral branches of the ventral divisions are shown in the thoracic region.

In the region of the anterior limb superficial tissues have been removed so as to expose the main structural features. Near the spinal column the trapezius and serratus anticus muscles are shown, the former being represented as semi-transparent. From the shoulder the greater portion of the deltoid muscle has been removed, from the upper arm the greater portion of the triceps, and from the forearm the greater portion of the extensor digitorum communis.

In the region of the leg the more superficial tissue has been removed so as to expose the skeletal, muscular, nervous and vascular apparatus.

The skeleton consists of hip-bone, femur, tibia and fibula, which are composed of embryonic pre-cartilage covered by a dense mesenchyme, and of a dense mass of tissue which represents the anlage of the ankle and foot.

The five lumbar and the first three sacral nerves enter into the formation of the lumbo-sacral plexus. From this arise the femoral nerve, which enters a mass of tissue that represents the extensor muscles of the thigh; the peroneal nerve, which gives off gluteal branches to the gluteal muscle mass, a posterior cutaneous branch, branches to the extensor musculature of the foot, and a peroneal branch, which extends a short distance along the fibula. The peroneal musculature has not yet become differentiated from the mesenchyme. At the posterior extremity of the plexus the pudic nerve may be seen.

The border vein empties into the femoral and sciatic veins. The sciatic artery is shown terminating in the extensor musculature of the foot.

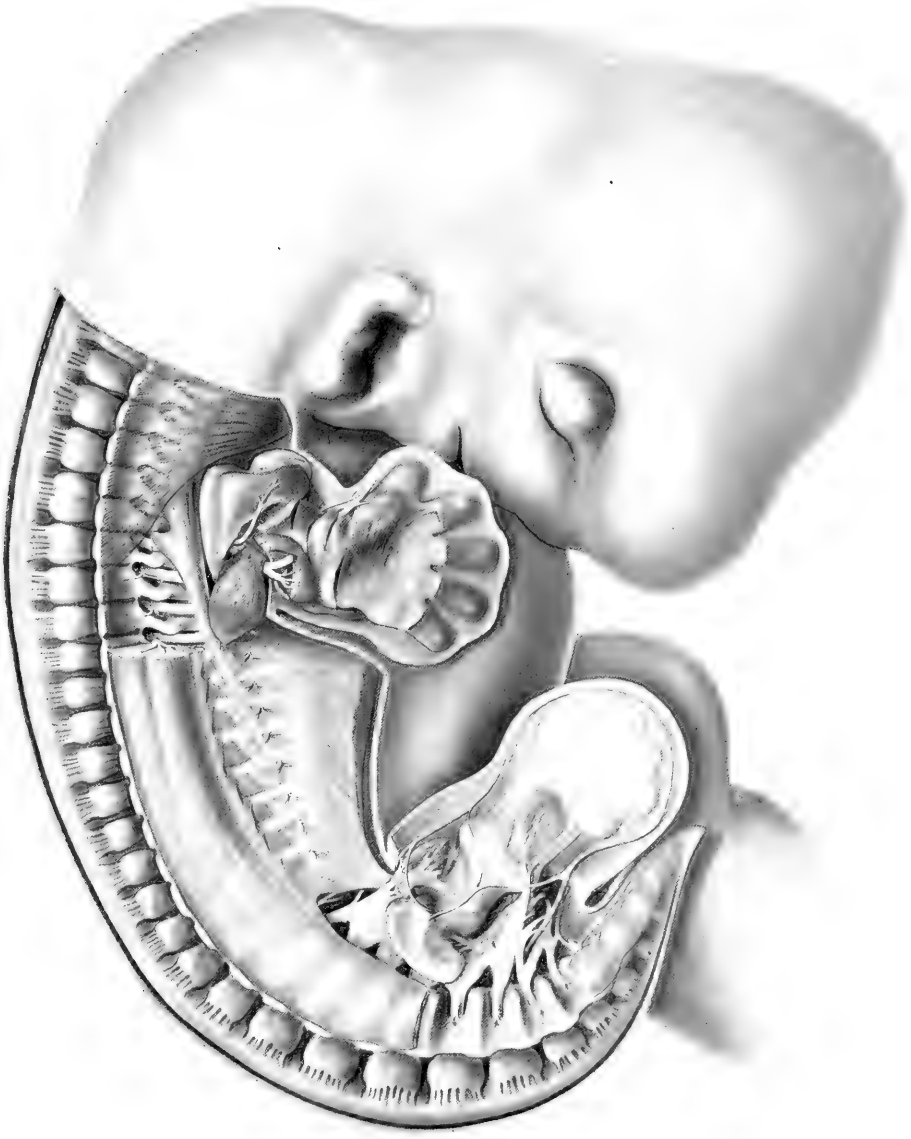


FIG. D.

PLATE V.

FIGURE E. Magnification about 15 d.

Drawing from a reconstruction of the regions of the arm, leg and body-wall of Embryo CIX; length, 11 mm.; age, about 5 weeks. In the arm region the cervico-brachial plexus, and the nerves and muscles of the arm and hand are exposed (see text, page 27); in the region of the body-wall the ribs, the spinal nerves, and the thoracic musculature (see text, page 26); [1] and in the region of the leg the lumbo-sacral plexus and the main nerves of the limb, the anlage of the limb musculature, the skeletal rudiment and the blood-vessels.

The five lumbar and the first three sacral nerves enter into the formation of the lumbo-sacral plexus. The twelfth thoracic nerve, however, sends a communicating branch to the ileo-hypogastric. From the first lumbar nerve arise the ileo-hypogastric nerve and a communicating branch to the lumbar plexus. From the lumbar plexus the femoral nerve may be seen passing behind the pubic process of the hip-bone into the extensor muscle mass. Just above the femoral nerve the genito-crural nerve arises from the plexus. Between the pubic and ischial processes of the hip-bone the obturator nerve passes forward into a mass of tissue which represents the adductor musculature. Below the ischial process the sciatic nerve passes into the limb, and from this the tibial nerve extends distally on the median surface of the skeleton of the leg and terminates in the flexor musculature of the foot. Along the course of the tibial nerve several muscle masses may be distinguished. These represent the perineal, obturator internus and quadratus femoris, ham-string and soleus-gastroenemus muscle masses. Posterior to the tibial nerve the pudic nerve arises. The sciatic artery passes in company with the tibial nerve, the obturator artery with the obturator nerve, and the femoral with the femoral nerve. The border, sciatic and femoral veins may be distinguished.

[1] A portion of the interosseal musculature has been removed near the tips of the first three ribs.

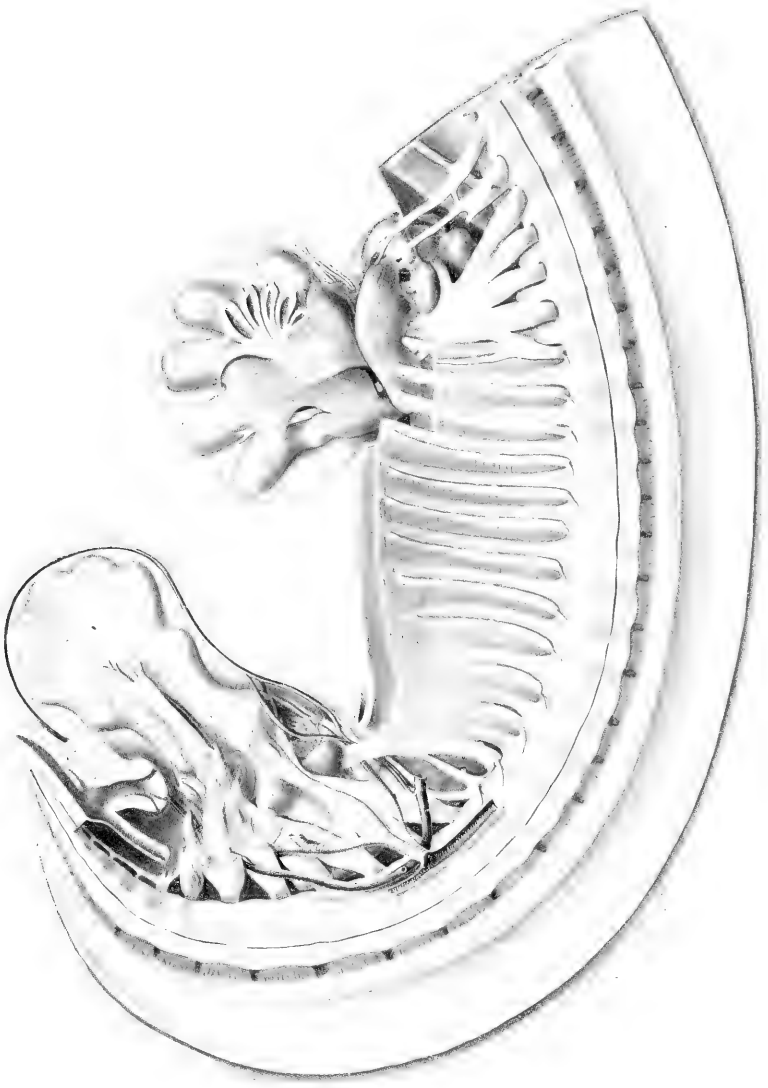


FIG. E

PLATE VI.

FIGURE F. Magnification about 10 d.

Drawing from a reconstruction of Embryo XXII; length, 20 mm.; age, about 7 weeks (see also Figs. G, H and I). From the arm, leg, body-wall, and the adjacent dorsal region the ectoderm and the superficial tissues have been removed. The muscles and nerves of the body-wall may be recognized readily from their likeness to adult structures. The shoulder muscles and the brachialis and triceps muscles of the upper arm are likewise plain. In the forearm the following muscles may be distinguished from above downwards: brachio-radialis, extensor carpi radialis longus et brevis, abductor pollicis longus, extensor pollicis brevis, extensor pollicis longus and extensor indicis proprius, extensor digitorum communis, extensor carpi ulnaris, and flexor carpi ulnaris; and in the hand the abductor minimi digiti. Branches from the circumflex and radial nerves may be seen.

In the posterior limb the sartorius and the extensor muscles (the vastus internus, rectus and vastus externus) may be seen above the femur. Between the femur and ilium the tensor vaginae femoris and the gluteus minimus, medius and maximus muscles may be seen. The biceps curves below the knee-joint. In the leg the tibialis anticus, extensor hallucis longus, extensor digitorum communis, and peroneus tertius muscles may be distinguished, and below the last the peroneal muscles. The middle and lateral cutaneous nerves lie over the thigh; the long saphenus, musculocutaneous and lateral saphenus lie exposed in the region of the leg and foot.

The perichondrium has been dissected away from the phalanges of the hand, leaving the cartilaginous cores visible. In the leg and foot the condensed mesenchyme or perichondrium surrounding the cartilages has been left intact.

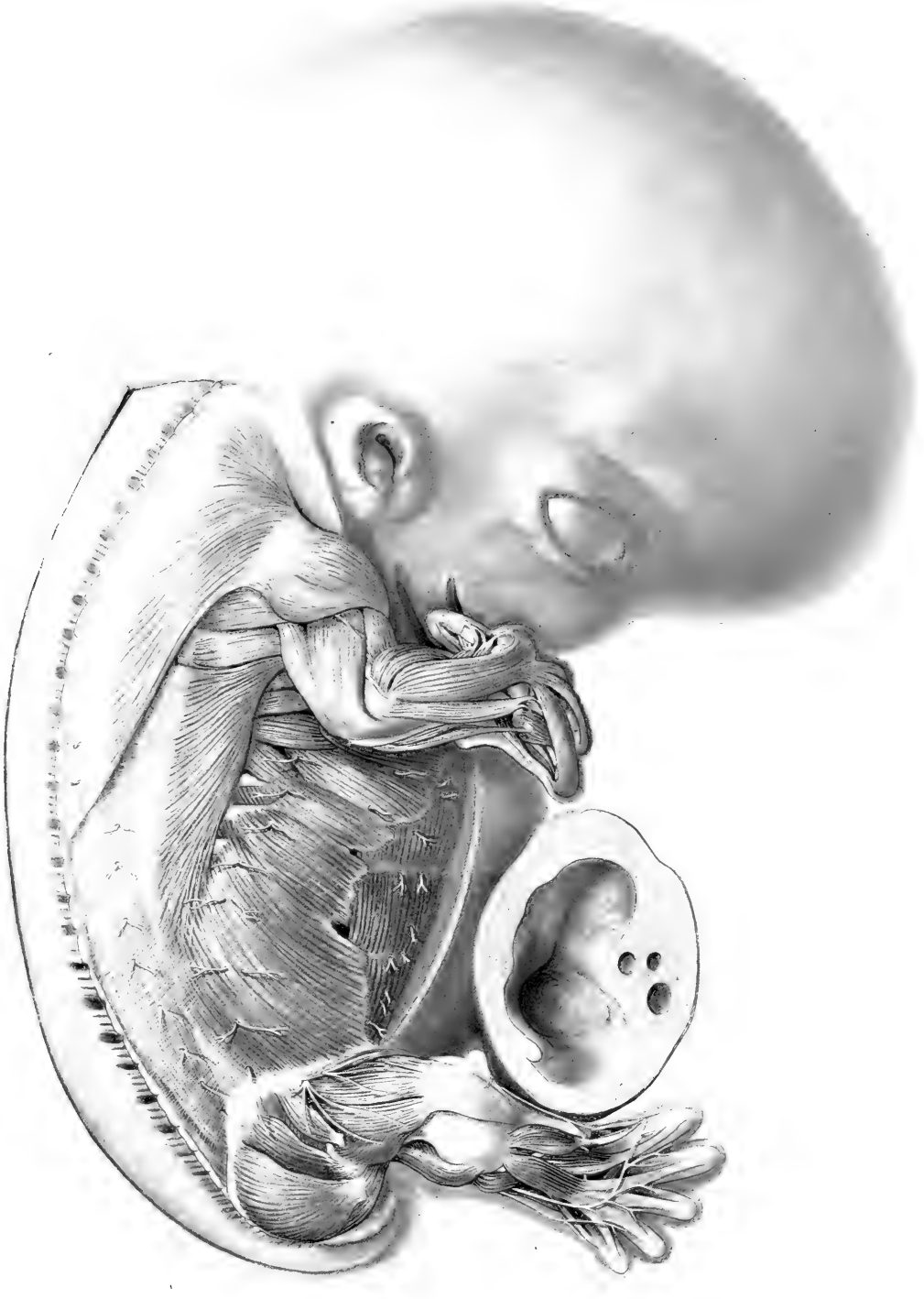


FIG. F.

PLATE VII.

FIGURE G. Magnification about 10 d.

Drawing from a reconstruction of Embryo XXII; length, 20 mm.; age, about 7 weeks (see also Figs. F, H and I). The left arm was not reconstructed, but has been drawn in part from the reconstruction of the right arm, in part from a photograph. In the region of the right arm the pectoralis major, biceps, coraco-brachialis, brachialis, brachio-radialis, extensor carpi radialis longus et brevis, the extensor communis digitorum and extensor carpi ulnaris; and the interossenis muscles may be seen. In the abdominal region the external oblique and rectus muscles are exposed. In the region of the posterior limb the adductor and ham-string muscles, and the tibialis anticus, the extensor pollicis longus and extensor digitorum communis muscles may be distinguished. The ventral tips of the thoracic nerves and the long saphenus nerve, and the tip of the anterior tibial nerve are shown.

The dense mesenchyme covering the cartilaginous parts of the skeleton is pictured intact.

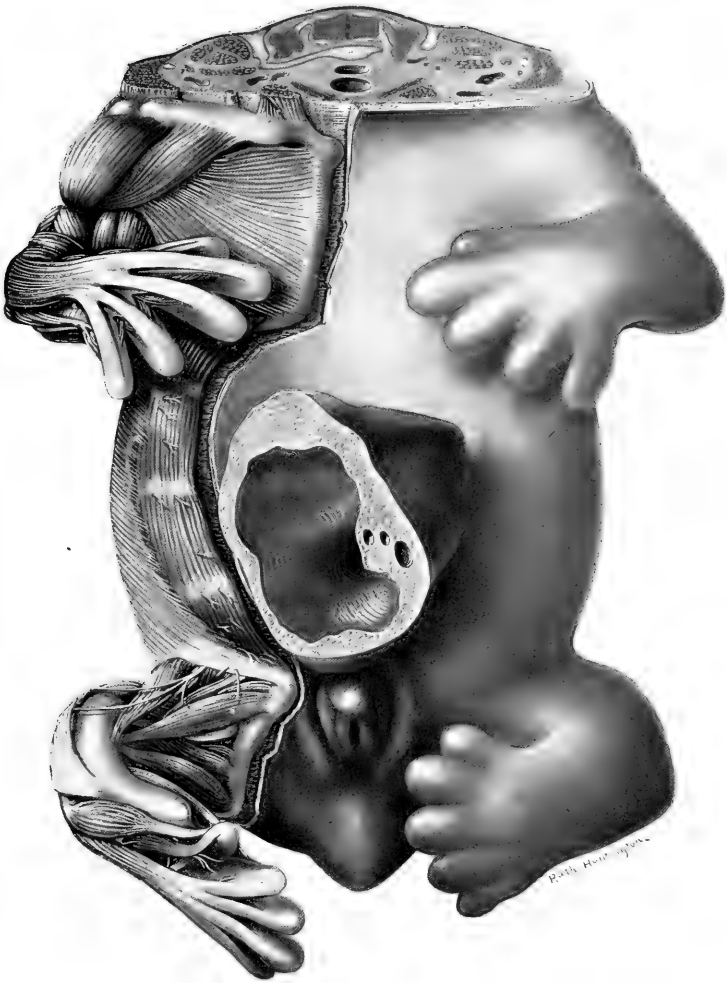


FIG. G.

PLATE VIII.

FIGURE H. Magnification about 10 d.

Drawing from a reconstruction of Embryo XXII; length, 20 mm.; age, about 7 weeks (see also Figs. F, G and I). The dorsal musculature has been removed. From the thoracic region all intrinsic muscles lateral to the internal intercostal muscles have been removed down to the 8th rib. The serratus anterior, however, has been left in position. The attachments of the external oblique musculature are shown. The rest of the external oblique muscle and a considerable portion of the internal oblique muscles have been removed. The thoraco-abdominal nerves are exposed. The most anterior nerve shown is the 7th cervical. The 7th cervical vertebra bears a rib-like process. The cartilaginous portions of the vertebræ and ribs of the first eight thoracic segments are represented. The ribs and vertebræ distal to this point are shown covered with a dense embryonic connective tissue.

The skeletal portions of the arm are drawn without the condensed mesenchyme or perichondrium which surrounds all the cartilages. Portions of the cartilages of the scapula, clavicle, humerus, ulnacarpus, metacarpus and phalanges are shown.

Most of the superficial muscles of the arm have been partially dissected away. Most of the deltoid, the infraspinatus and teres minor, the teres major and latissimus dorsi, the triceps and anconeus, the extensor digitorum communis and the extensor carpi ulnaris muscles have been dissected away except for their attached ends which can be readily recognized. The senatus anterior, supraspinatus brachialis, brachioradialis, extensor carpi radialis longus et brevis, supinator brevis, abductor pollicis longus, extensor pollicis brevis, extensor pollicis longus and extensor indicis muscles are left intact. The intrinsic muscles of the hand as well as the insertions there of the muscles of the forearm have been entirely cut away.

The suprascapular, circumflex and radial nerves with their main branches are shown.

In the posterior limb portions of the rectus and sartorius muscles have been removed so as to expose the chief branches of the femoral nerve; and portions of the tensor vaginæ femoris, gluteus medius and gluteus maximus muscles, so as to expose the chief branches of the gluteal nerves. In the leg a portion of the extensor digitorum communis muscle has been removed so as to expose the extensor hallucis longus and the extensor digitorum brevis muscles, and the distribution of the anterior tibial nerve. Portions of the peroneal muscles have been removed so as to expose the distribution of nerves to these muscles.

In the anterior limb the cartilaginous portions of the skeleton are represented except at the joints, where some condensed tissue is pictured. In the posterior limb is shown the condensed tissue which incloses the cartilaginous portions of the skeleton.

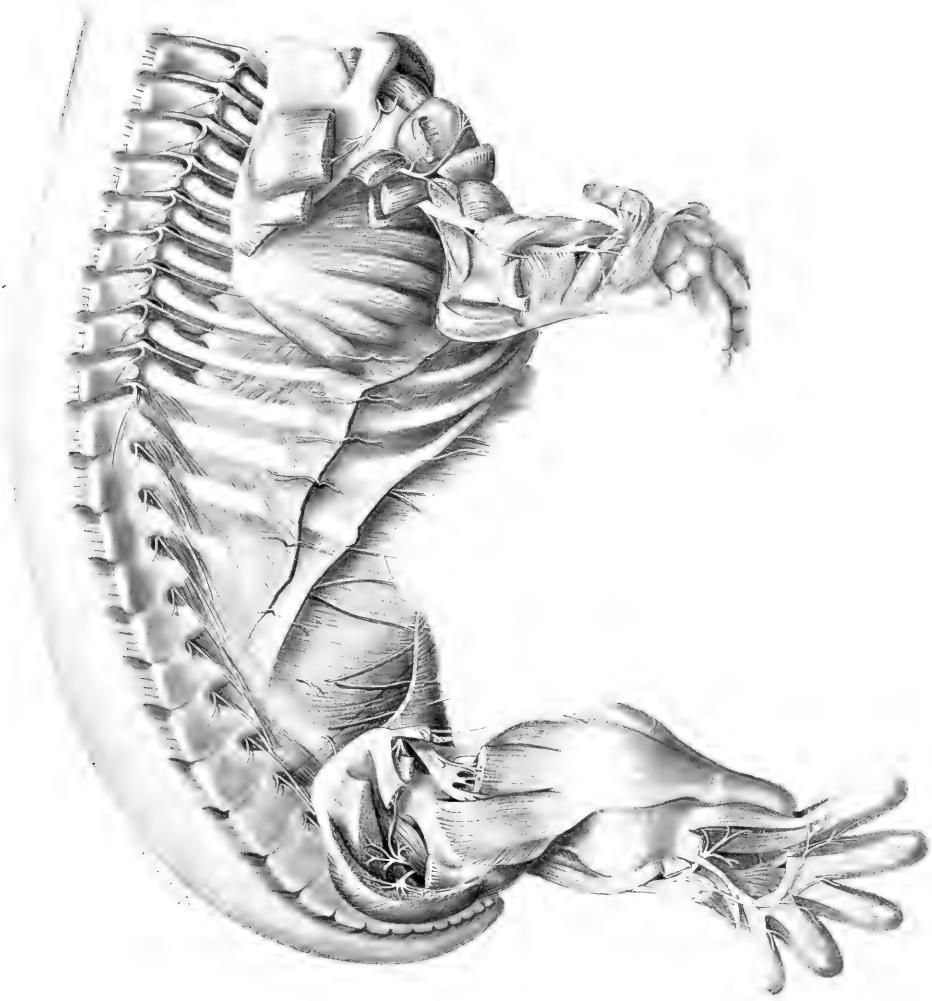


FIG. H.

PLATE IX.

FIGURE I. Magnification about 12 d.

Drawing from a reconstruction of Embryo XXII; length, 20 mm.; age, about 7 weeks (see also Figs. F, G and H). The thoracic, abdominal and pelvic viscera have been removed. The attachment of the diaphragm to the body-wall is shown. The intrinsic muscles and nerves of the thorax, abdomen and pelvis are shown intact, and may be readily distinguished by their relative positions.

The ventral ends of the upper four ribs and the median end of the clavicle are not shown.

In the region of the shoulder and upper arm the deltoid, biceps, brachialis, coracobrachialis and subscapular muscles are shown intact. The attached ends of the pectoral muscles may be seen. In the forearm the following muscles may be distinguished from above downwards: brachio-radialis, pronator teres, flexor carpi radialis, palmaris longus, flexor digitorum sublimis and flexor carpi ulnaris. The brachial plexus is shown together with the main nerves of the arm. Some of the interossei muscles are shown in the hand.

The brachial plexus arising from the 5th to 8th cervical and 1st thoracic nerves is seen. The relatively large size of the nerves and plexus is at once noticed. The plexus itself forms a closely packed mass of fibers in which it is just possible to distinguish the position of the three main cords from which the principal nerves of the arm arise. The posterior cord is not visible in this figure. The suprascapular, small nerve to the subclavious, branch to the pectoral muscles, musculocutaneous, median, ulnar, and internal cutaneous nerves are seen arising from the plexus.

In the region of the posterior limb the psoas muscle is shown cut away over the lumbar plexus. The sartorius and vastus medius muscles may be seen above the femur. In the region of distribution of the obturator nerve the belly of the gracilis muscle has been removed so as to expose the adductor muscles. The bellies of the semi-membranous and semi-tendinosus muscles have been removed so as to expose the sciatic nerve, below which the long head of the biceps may be seen. In the leg the gastroenemeus, soleus, popliteus muscles, and the flexors of the toes may be distinguished. The lumbo-sacral plexus arises from the 12th thoracic to the 3d sacral spinal nerves. The inguinal nerve arises from in front of the first lumbar nerve, the genito-crural from in front of the second, and the lateral cutaneous from a point just above the region where the obturator and femoral nerves are given off. From the lumbar plexus a large nerve bundle passes into the psoas muscle. After passing Pouparts ligament the main trunk of the femoral nerve may be seen below the sartorius muscle. The long saphenus nerve may be traced to the ankle. The main branches of the obturator nerve are shown. The pudic nerve may be seen passing out between the great sacro-iliac ligament and the levator ani muscle, the posterior cutaneous nerve is given off on the lateral side of sacro-iliac ligament. The main branches of the tibial nerve in the leg and foot may be readily followed.

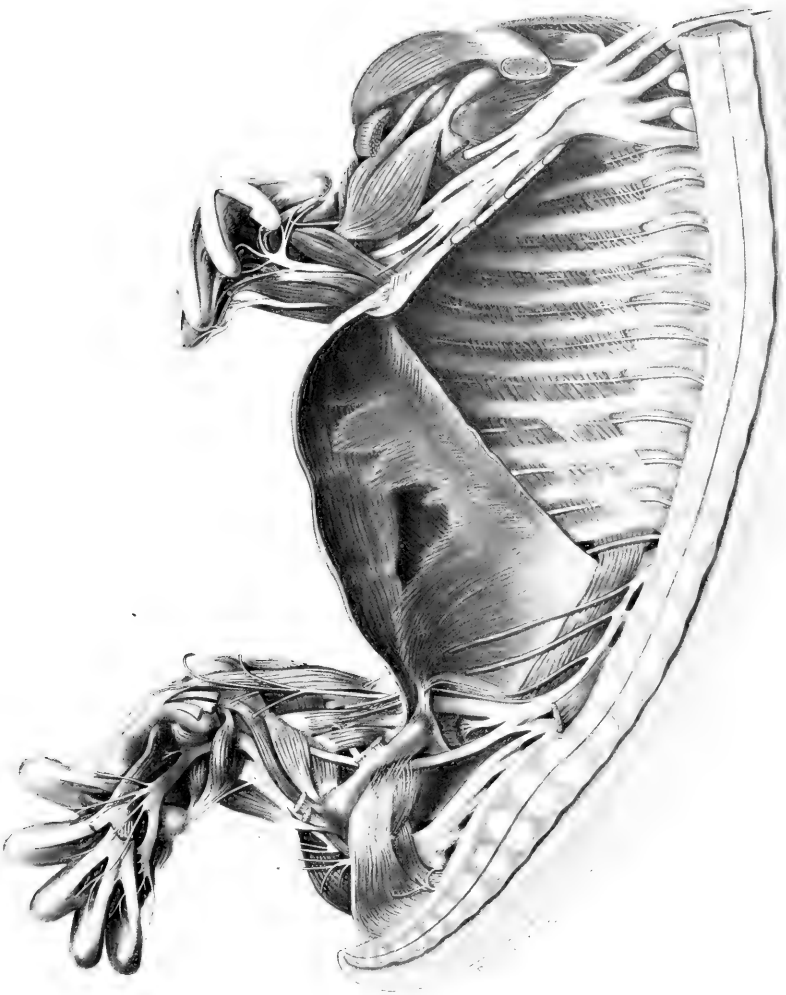


FIG. I.

THE INTRALOBULAR FRAMEWORK OF THE HUMAN SPLEEN.

BY

PRESTON KYES, A. M., M. D.

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WITH ONE TEXT FIGURE.

The coarser framework of the human spleen divides the organ into small masses of parenchyma—the lobules, each having an afferent artery near its center and the larger efferent veins at its periphery.

The finer framework of the spleen subdivides each lobule and supports the individual spleen cells and smaller blood-vessels contained therein. The finer framework is an extension within the lobule of the coarser framework of the trabeculæ and is here indicated as the intralobular framework.

HISTORY.

Oesterlen,[1] writing in 1843, mentioned, without description, a substance between the individual cells of the spleen pulp.

Atto Tigri,[2] in 1853, described as a constant element of structure an intercellular network giving support to the spleen cells.

Billroth,[3] in 1857, confirmed the presence of this intercellular framework, and described its minute structure more in detail. From a comparative study of the vertebrata, he concluded that the framework was formed by stellate cells whose anastomosing processes appeared as fibers connecting the cell bodies proper with one another. Thus, according to Billroth, the cell bodies appeared as local expansions of the fibrils, each containing a definite nucleus. From the spleens of amphibia he isolated stellate cells that he asserted were the branched cells forming the intralobular framework. From the human spleen, however, he could not isolate the corresponding cells, and stated that their demonstration *in situ* was more difficult than in lower forms because of their relative infrequency.

[1] Oesterlen, F.: *Beiträge zur Physiologie des gesunden und kranken Organismus*, Jena, 1843.

[2] Tigri, A.: *Schiarimenti sulla struttura e sulla funzione della milza*, *Gazz. med. ital. feder. tosc.*, Firenze, 2 s., T. III, 1853, pp. 25–27.

[3] Billroth, T.: *Beiträge zur vergleichenden Histologie der Milz*, *Arch. f. Anat., Physiol., u. wissensch. Med.*, Berl., 1857, S. 88–108.

Henle,[4] in 1859, questioned the findings of Billroth and denied the existence of nuclei within expansions of the fibers. He described the intralobular framework as composed, not of anastomosing cells, but of fibrils similar to those of tendon.. These fibrils crossing one another in all directions and at varying angles were, according to Henle, direct continuations of the white fibrous tissue of the trabeculæ. He further described certain circular and spiral fibrils anastomosing with one another to encircle the capillary veins.

W. Müller,[5] in 1865, corroborated the findings of Henle as to the fibrillary character of the intercellular framework. He further described a finely granular intercellular ground substance within which he considered the individual fibrils to be imbedded.

Oppel,[6] in 1891, employing precipitation methods, was able to determine with some accuracy the general distribution of very delicate intercellular fibrils within the pulp cords and Malpighian follicles of the human spleen. Such fibers he termed "Gitterfasern," but he gave no data as to their intimate structure.

Mall,[7] in a series of contributions, commencing in 1888, established a lobule as the unit of structure of the dog's spleen and determined that its framework is composed largely of connective tissue differing from both the white fibrous and yellow elastic forms. The component fibrils of this tissue he found to be devoid of nuclei and everywhere anastomosing with similar fibrils to form a delicate but resistant network. Unlike elastic tissue, these fibrils resisted pancreatin digestion and differed from white fibrous tissue in their extensive branching and anastomosis. To this tissue he applied the specific term "reticulum." The most delicate framework within the pulp cords he described as a distinct variety of reticulum not resisting pancreatin digestion. The reaction of the reticulum within the Malpighian follicle he did not determine.

METHODS.

In studying the framework of fifteen human spleens, I have employed the so-called "destructive" methods, the removal of the spleen pulp by maceration and digestion permitting inspection of the isolated frame-

[4] Henle: *Zeitschr. f. rationelle Medizin*, III Reihe, Bd. VIII, S. 201–230.

[5] Müller, W.: *Ueber der feineren Bau der Milz*, Leipzig, 1865.

[6] Oppel, A.: *Ueber Gitterfasern der menschlichen Leber und Milz*, *Anat. Anz.*, Jena, Bd. VI, 1891, S. 165–173.

[7] Mall: *Anat. Anz.*, 1888. *Abhandl. d. K. S. Ges. d. Wiss.*, Bd. XVII, 1891. *Johns Hopkins Hospital Reports*, Vol. I. *Johns Hopkins Hospital Bulletin*, Nos. 90–91, 1898. *Zeitschift f. Morphologie u. Anthropologie*, Bd. II, 1900.

work. Pancreatin digestion was used as suggested by Mall and Spalteholz. According to Mall's method, sections of fresh tissue are digested in the following solution:

Pancreatin, 5 gms.; bicarbonate of soda, 10 gms.; water, 100 ccm.

The resulting specimen is washed by shaking in a volume of water and then allowed to dry on the slide. To stain the section, a few drops of the following solution are allowed to dry on the specimen:

Picric acid, 10 gms.; absolute alcohol, 33 ccm.; water, 300 ccm.

The specimen is then immersed for one-half an hour in the following solution:

Acid fuchsin, 10 gms.; absolute alcohol, 33 ccm.; water, 66 ccm.

Upon removal, the tissue is washed with the picric acid solution, dehydrated, cleared in xylol and mounted in balsam.

The method of digestion suggested by Spalteholz has been of especial service because of the support given to the delicate framework by the glass slide and because of the accuracy of the control. According to this method the tissue to be digested is fixed in a one per cent solution of mercuric chlorid in thirty-three per cent alcohol for twenty-four hours. Each succeeding twenty-four hours the tissue is transferred to fresh alcohol increased ten per cent in strength, until absolute alcohol is reached. The tissue is imbedded in paraffin and cut in serial sections from $6\frac{2}{3}$ to 20 microns thick. The sections to be digested are fixed to the slide by the water method and the process continued as follows:

> Remove the paraffin with xylol; wash in absolute alcohol; immerse in benzine at 38° C. for 24-36 hours; wash in absolute alcohol followed by 95 per cent alcohol; wash in water five minutes; digest in a solution of pancreatin at 38° C. for 12-48 hours; wash in water 10 minutes; stain with iron hæmatoxylin; mount in balsam.

By substituting acetic alcohol[8] for the graded alcohols, the time required for fixing may be reduced from several days to a few hours.

The pancreatin solution was employed as follows:

> Pancreatin ferment (Grübler), 1 part; sodium bicarbonate, 20 parts; thymol, 10 parts; distilled water, 10,000 parts.

The digestion of hardened tissue in bulk was employed as follows: Small pieces of tissue were hardened 4-12 hours in acetic alcohol and dehydrated in absolute alcohol. They were then extracted with ether for 10-14 days. The tissue was then digested in an aqueous solution

[8] Carnoy: *La Cellule*, T. III, 1886.

of pancreatin and washed in water. The resulting specimens were in some cases stained, imbedded and sectioned; in others immersed in glycerin and studied with a low-power stereoscopic microscope. The thick sections thus obtained afforded most instructive pictures in three dimensions.

Controls of the digested specimens were stained with orcein, Weigert's elastic tissue stain, hæmatoxylin and eosin, and the connective tissue stains of Mallory and Van Gieson. In searching for elastic fibers within the lobule the so-called differential stains of Weigert, Mallory and Unna-Tänzer were employed.

Digested specimens were stained by immersing them in a five per cent solution of the ammonio-sulphate of iron for 12-18 hours, and subsequently removing them to a five per cent aqueous solution of hæmatoxylin for four hours. By differentiating such specimens in a one per cent solution of acetic acid a sharp and intense black stain was obtained. Weigert's elastic tissue stain was also used to stain digested specimens.

Dilute aqueous solutions of potassium hydrate, acetic and hydrochloric acids were employed in testing and differentiating the connective tissues of the framework.

DISCUSSION.

By macerating and washing fresh human spleen tissue it is possible to remove the parenchyma cells and expose a framework of blood-vessels and anastomosing fibers that outlines the general form of the specimen in all dimensions. This is the coarser framework of the spleen formed by the trabeculæ and is of uniform density throughout the organ. Its meshes, incompletely marked off by the surrounding fibers, average 20 microns in diameter and are the compartments occupied by lobules. In a specimen thus prepared each compartment appears as a vacuole, its outline alone being indicated.

If, however, spleen tissue be hardened in alcohol, thoroughly extracted with ether, and the parenchyma entirely removed by pancreatin digestion without mechanical injury, there is preserved in addition to the coarser framework a delicate network of fibrils within each individual compartment—the intralobular framework. (Fig. 1, C.)

The delicate fibrils composing the intralobular network vary from 1 to 5 microns in diameter. They branch and anastomose in all directions to form a network with meshes from 16 to 40 microns in diameter. The fibrils are directly continuous with those of the coarser interlobular framework at the periphery of the lobule

and with the sheaths of the blood-vessels within the lobule. The network is devoid of nuclei and the picture is that of a continuous system of branching and anastomosing threads of fairly uniform caliber. The extent of an individual fibril cannot be determined.

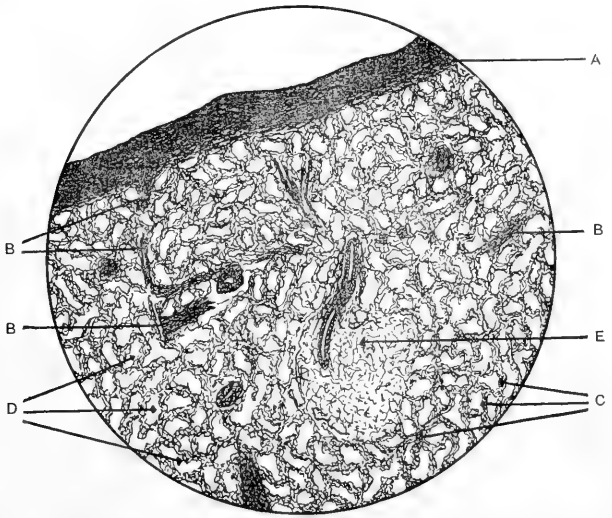

FIG. 1.—Congested human spleen; digested with pancreatin; stained with iron hæmatoxylin; Zeiss, Obj. AA, Oc. 2.

A. Capsule.
B. Trabeculae of coarser framework.
C. Intralobular framework.
D. Intralobular capillary veins [Billroth].
E. Malpighian follicle with afferent artery.

The network extends throughout the entire lobule between the capillary veins and in a modified form within the Malpighian follicles. Thus the arrangement is such as to outline in the digested specimens all the structures appreciable in the undigested specimens. The general distribution of the network throughout the lobule is interrupted by irregularly circular channels averaging 0.18 mm. in diameter. These channels freely communicate with one another, and in thin specimens appear as circular and oval lacunæ surrounded by the framework of the pulp cords. They represent the lumina of the intralobular capillary veins first described by Billroth. (Fig. 1, D.)

The arrangement of the fibrils immediately surrounding the capillary veins is so modified as to form a definite framework limiting the venous spaces. Continuing from the general network of the pulp cords

the fibrils encircle the venules and anastomose with one another to form a series of circular and spiral rings completely surrounding the lumen. They are an integral part of the framework of the pulp cords, modified only as to their distribution. Encircling and limiting the venous spaces the spirals are not compactly apposed to one another, but separated by intervening spaces of varying width. They are identical with the fibrils first described by Henle as white fibrous tissue and by more recent writers as elastic fibers. The nature of the fibril *per se* will be discussed below.

In fortunate preparations the intralobular framework may be seen to extend throughout the Malpighian follicle and become continuous with the coats of the afferent artery. (Fig. 1, E.) The fibrils are much more delicate and fewer in number than those of the pulp cords. The meshes are larger and the network is uninterrupted by the spaces representing the capillary veins. There is no membrane or condensation of the framework at the periphery of the follicle to mark it off from the surrounding portion of the lobule, the only transition being from the coarser to the more delicate network.

In all their reactions the component fibrils correspond to reticulum as described by Mall. They differ from elastic fibrils in that they are not digested with pancreatin ferment and are resistant to weak acids and alkalies. Unlike white fibrils, they continually branch and anastomose. Their reaction with pancreatin is constant, and in specimens subjected to the enzyme for forty-eight hours the framework remains intact where sufficiently protected from mechanical injury. The fibrils within the Malpighian follicles, because of their extreme delicacy, are easily broken away in the process of washing, and in a majority of specimens this portion of the framework is wanting. In fortunate preparations, however, this framework is present, intact, after the continued action of the enzyme.

The staining reaction of these fibrils is intermediate between that of elastic and white fibrous tissue. By prolonging the time of staining or by heating, reticulum fibrils may be tinted and in cases somewhat deeply stained by the so-called specific elastic tissue stains of Weigert, Mallory and Unna-Tänzer. Sections of human spleen from which all elastic tissue has been removed by digesting forty-eight hours in pancreatin, may be tinted a deep purple by staining two hours in Weigert's elastic tissue stain. By continuing the staining for three hours at 38° C. the intensity of the color is much increased. The reaction is not the same as that with elastic fibrils, the intense black color never being attained with reticulum as with elastic tissue. Likewise with the orcein

method, the fibrils may be stained brown and with Mallory's stain a blue-black. I have not been able to obtain similar reactions with the white fibers of tendon.

Such staining reactions would seem to be responsible for the continued descriptions of elastic fibrils within the pulp cords, the walls of the capillary veins and the Malpighian follicles. Fibers within all of these structures may be brought into view by overstaining with the so-called elastic tissue stains. The staining reaction of such fibers, however, is not that typical of the elastic fibers seen within the trabeculæ and the arterial walls, and unlike elastic fibers, they resist pancreatin digestion. I quite agree with Höhl [9] that the fibrils within the walls of the capillary veins of the human spleen described by v. Ebner [10] as elastic tissue cannot be such, since they resist pancreatin digestion. In addition their staining reactions are those of reticulum found elsewhere.

SUMMARY.

1. Within the lobule of the human spleen there is a delicate network of fibrils continuous throughout the pulp cords and the Malpighian follicles.

2. The fibrils of this entire network are reticulum in the sense of Mall.

3. The fibrils encircling the capillary veins are an integral part of this reticulum network and are not elastic tissue.

4. The so-called specific elastic tissue stains yield a positive reaction with reticulum as well as with elastic fibers.

[9] Höhl, E.: *Anat. Anz.*, Bd. XVII.
[10] v. Ebner: *Anat. Anz.*, Bd. XV.

STUDIES ON THE NEUROGLIA.

BY

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Our present conception of the structure of neurogliar tissue is based in the main on results obtained by the employment of two fundamentally different methods, each of which is regarded by its supporters as a differential stain for neuroglia. We refer here to the chrome-silver method of Golgi, for many years the only method at our disposal by means of which the structural elements of this tissue could be brought to light. To this method we are indebted for the results obtained by Golgi, v. Lenhossék, v. Kölliker, Ramon y Cajal, Retzius, Sala y Pons, Van Gehuchten, Eurich, Reinke and others in their investigations of the structure and histogenesis of the neuroglia. The other method was the one fully described by Weigert in 1895 in his large monograph on the structure of neurogliar tissue, giving the results of seven years' labor in this field. Almost simultaneously appeared Mallory's publication giving an analogous method. In the Weigert and Mallory methods, the staining is the result of a chemical differentiation of the neuroglia fibers. Their results have been corroborated by Pollack, Krause and Aguerre working with normal tissues and by Taylor, Storch and Bonome in their study of pathological tissue and new growths. Quite recently Benda has published a differential stain for neuroglia, which seems destined to become very useful.

All recent writers on neurogliar tissue have called attention to the apparently contradictory results obtained by those observers working with the chrome-silver method as compared with the recorded observations of investigators who have used the more modern differential stains, and a glance at the more recent literature is sufficient to convince one that a classification of the current views of the structure of neurogliar tissue may with propriety be based on the methods used in the investigation of the tissue. It is, however, not our purpose at the present time to extend this controversy, and a repetition of what has been previously stated in several of the discussions of neuroglia literature seems uncalled for; it will therefore be entered upon only to the extent necessary to present our own observations as clearly and succinctly as possible.

As is now very generally known, the chrome-silver method shows the neurogliar tissue to consist of cellular elements possessing a varying number of fibrillar processes. These structural elements have been designated by v. Lenhossék by the very appropriate name of *astrocytes*, and are subdivided into (a) astrocytes with long processes, found in the white and grey matter, and (b) astrocytes with short processes, found only in the grey matter. In these few words we have endeavored to reflect, as well as possible in so short a space, the views of the majority of the early observers who have used the chrome-silver method in the study of neuroglia. V. Kölliker, while agreeing in the main with the above statement of the structure of the neuroglia, describes the fully-developed astrocyte, which he terms a *Golgi-cell*, as consisting of two portions—a cell-body containing the nucleus which is intimately associated with a *cell-plate*, from which the cell processes arise. He suggests the hypothesis that "a Golgi-cell with a portion of its protoplasm develops a cell-plate from which the cell processes arise; this plate originally and as long as the processes possess the power of growth is intimately connected with the nucleated portion of the Golgi-cell; in many instances, however, the cell-plate attains a different consistency and perhaps also a different constitution and, under certain conditions, may sever its connection with the nucleated portion of the Golgi-cell." Andriezen, who has used the chrome-silver method in studying the neurogliar tissue of the brain cortex, recognizes two varieties of neuroglia cells. One of these, which he has designated as the *neuroglia fiber cells*, corresponds in the main with the astrocytes with long processes described by other writers. It should, however, be stated that this observer was able to make out in certain of his preparations that not all neuroglia fibers are processes of cells, but that many of them "pass right through the cell body." The other variety of neuroglia cell is described by him under the name of *protoplasmic glia cell*, and has stout, coarse and very shaggy processes, which vary very greatly in size. These latter cells are said to be of mesoblastic origin. Reinke, who, in his study of neuroglia tissue, has made use of a modified chrome-silver method (after obtaining a chrome-silver precipitate, he dehydrated and then imbedded his tissue in paraffin, fixed the sections with albumin fixative and then stained them with Heidenhain's hematoxylin and counterstained them in eosin), has obtained results which deserve especial mention. His conclusions are "that the neurogliar tissue of the white substance of the human cord consists of (1) cells and (2) fibrils. The cells possess numerous processes, some of which are branched and which run in part transversely and in part obliquely; the majority of

them, however, run vertically, that is, parallel to the nerve-fibers. These processes are well stained by the chrome-silver method. The fibrils differ morphologically, physically and chemically from the cell-processes. They are, however, developed from the protoplasm of the cells and lie partly in and partly on the protoplasm and have a direction which in the main is opposite to that of the cell processes. For the most part these fibrils, the length of which is unknown, are emancipated from the cell-bodies. The fibrils differ in thickness and probably do not anastomose. These are the fibrils which are so clearly brought out by the Weigert method."

Erik Müller's "Studies on Neuroglia" may also be mentioned in this connection. His observations were made on tissues taken from amphioxus, myxine, acanthias, and from teleosts; they were fixed by the Golgi method and then stained after Heidenhain's iron-lack-hematoxylin. In all of these forms he finds the neuroglia made up of cells and fibers, which are, however, in such relation to each other that all the fibers may be regarded as processes of cells.

As may be seen from the above brief summary of neuroglia literature giving accounts of observations made with the chrome-silver method, all observers who have used this method, even when such method has been subjected to special modifications, have reached the conclusion that the neuroglia is composed of cellular elements and cell processes—neuroglia-cells and neuroglia-fibers, the latter being the processes of the former. It is true that Andriezen found certain astrocytes in which the neuroglia fibers passed through the cell-body, and that v. Kölliker regards himself justified in describing a cell-plate with cell-processes, which may under certain conditions become separated from the nucleated portion of the neuroglia cell, and that Reinke believes that he has harmonized the conflicting views by his discovery of two varieties of neuroglia fibers, the one being processes of neuroglia cells and the other developed from the protoplasm and in part at least emancipated from such protoplasm; yet a careful study of the account and figures given by Andriezen and v. Kölliker does not reveal sufficient evidence to indicate that these observers regard the neuroglia fibers as other than processes of the neuroglia cells, and Reinke's statement will need further confirmation with methods other than a chrome-silver precipitate before they can be accepted as established. At this date it does not seem necessary to enter into a consideration of the chrome-silver method as such, as its advantages and disadvantages have been the subjects of frequent consideration and are now very generally known; suffice it to say that we agree with Taylor when he states that

"A method which colors by precipitation is *a priori* incapable of giving us the information which we require; it must of necessity be confusing in its pictures of structural detail."

We may now turn our attention to the observations of investigators who have employed a differential chemical stain in their study of neuroglia. Weigert, whose comprehensive work gave a new impetus to the study of neuroglia, summarizes his results in the following statements: "The neuroglia fibers, which have been hitherto regarded as processes of Deiters' cells, differ chemically from the protoplasm of these cells. This difference in the chemical constitution of the 'cell-processes' is apparent in the immediate vicinity of the cell nucleus, as well as at some distance from it. The majority of the so-called cell processes are not cell processes since two such apparent processes form a continuous fiber, which is in no way interrupted by the cell body, as would be the case were they true processes arising individually from the cell body. In a word, there is no question here of cell processes or cell extensions, but of fibers which are fully differentiated from the protoplasm." Weigert describes two types of nuclei of neuroglia cells—large, vesicular nuclei the chromatin of which has a granular appearance, and smaller ones in which the chromatin forms a deeply-staining, homogeneous mass, with transition forms between the two varieties. The large vesicular nuclei often show a definite relation to the neuroglia fibers, in that they form centres, over which and around which the neuroglia fibers pass, simulating in a most characteristic manner the astrocytes of other investigators. Numerous free neuroglia cell nuclei, notably of the smaller, deeply-staining variety, which bear no special relation to the neuroglia fibers, are also found.

Mallory, working with his own method, obtained results which were practically identical with those obtained by Weigert. Although these two investigators worked independently, their results were published almost simultaneously. Pollack in a short note confirms Weigert's statements and calls attention to the importance of his differential neuroglia stain and the results obtained. Aguerre, after discussing the recent neuroglia literature and calling attention to the superiority of Weigert's neuroglia stain and corroborating his observations, discusses at some length the shape and structure of the neuroglia cell nuclei as found in the human spinal cord, stating that these nuclei vary much in shape and size and are often polymorphous. He classifies the neuroglia cell nuclei according to their size as follows: (1) small nuclei, 3μ to 4μ, to which variety the small deeply-stained nuclei belong; (2) medium-sized nuclei, 6μ to 8μ, the smaller nuclei of the

vesicular variety; (3) large nuclei of the vesicular variety, often polymorphous and measuring as high as 14μ. Krause and Aguerre, in another communication, describe at some length the distribution of the neuroglia tissue in the human spinal cord. Attention should also be drawn to the fact that these two observers have been able to stain the neuroglia in apes and half-apes, after having slightly modified the Weigert method. Krause has given a full account of the neuroglia in the spinal cord of the ape, in which he describes small, deeply-staining nuclei of neuroglia cells and larger often polymorphous, vesicular nuclei, having only a small amount of chromatin. He finds the majority of the neuroglia fibers fully differentiated and many of them relatively thin. Eurich in his last publication places himself in accord with Weigert's view on the structure of the neuroglia and advances theoretical reasons for accepting the same. It would lead beyond the limits of this paper to do more than mention the observations of Taylor, who worked with the Mallory method, and Storch and Bonome, who used the Weigert method in their study of glioma and gliosis and of the behavior of the neuroglia in certain pathological conditions of the central nervous system of man. As pertains to the structure of the fully-developed neuroglia tissue, their published results confirm in the main the views expressed by Weigert and Mallory and others who have used these methods. Yamagiwa has recently described a new stain for neuroglia which consists of a modification of Ströbe's differential axis-cylinder stain. As a result of observations made with this method, he is led to conclude that the neuroglia fibers are differentiated intercellular structures, which are, however, not entirely or not in all instances completely separated from the neuroglia cells. Mention may also be made of a somewhat crude method described by Whitwell, by means of which he aims to differentiate the neuroglia fibers. Sections from hardened tissues are treated for a few seconds with a hot concentrated solution of caustic potash, are then rinsed in water and allowed to desiccate on the slide. When dry, the sections are covered with a cover glass. In such preparations examined with a moderate magnification, but with good illumination, a dense feltwork of fibrils may be seen. Whitwell regards these fibrils as neuroglia fibers and states that "they show no evidence of being direct processes of cells and do not appear to branch and form a complete basket network for each element in the nervous tissues, including the blood-vessels." Benda has recently described a differential neuroglia stain which will be given fuller consideration presently and will therefore receive no further mention at this time. From the foregoing account it may be seen that those

observers who have used differential neuroglia stains in their study of this tissue agree in regarding the neuroglia fibers, not as cell processes, since they are readily differentiated from the protoplasm of neuroglia cells, from which they differ in chemical constitution and physical properties, as is shown by these staining methods, but as an intercellular substance emancipated from the protoplasm of the neuroglia cells.

One of the disadvantages of both the Weigert and Mallory neuroglia stains is the fact that these stains can be used only on human tissue; furthermore, owing to the fact that the neuroglia fibers break down very readily, as was pointed out by Virchow many years ago, it is necessary to have at one's disposal very fresh tissue in order to obtain satisfactory staining of the neuroglia. Krause and Aguerre, as has been previously stated, were able by careful manipulation of the Weigert method to obtain successful differential staining of the neuroglia in apes and half-apes; this, however, is the only instance, so far as I have been able to ascertain, in which a successful differential staining of the neuroglia in vertebrates other than man has been obtained. Weigert and Mallory both admit that their neuroglia staining methods are useful only on fresh human tissue. The difficulty of obtaining very fresh human tissue no doubt accounts for the fact that we have so few confirmatory observations of the views of Weigert and Mallory concerning the structure of the neuroglia. The fact that it is often difficult to obtain fresh human tissue and the further fact that these methods cannot be used to stain the neuroglia of animals no doubt explains in part the apparent hesitancy to accept their results in place of the results obtained by the chrome-silver method, by means of which, as is well known, neuroglia tissue may be stained in the central nervous system of all vertebrates, whether embryonic or adult tissue is used. The reason why the Weigert and Mallory stains are selective for human neuroglia only is difficult to explain and must be ascribed to some slight difference in the chemical composition of the neuroglia fibers of man and other vertebrates, in which case we may look upon these staining methods as so highly differential as to be applicable to the staining of human neuroglia only. In the hope that by modifying these methods a procedure might be found by means of which the neuroglia of animals might be stained, I spent much time in experimentation. All of my endeavors, however, proved fruitless so long as I confined my attention to the Weigert and Mallory methods. Much more satisfactory results were, however, obtained after the writer became familiar with the Benda differential neuroglia stain, published in September of last year. Benda was led to continue his endeavors to discover a satisfactory stain for

neurogliar tissue, partially interrupted by the appearance of Weigert's large monograph, because the Weigert method did not seem to possess the certainty ascribed to it by its discoverer. I am not aware that Benda is familiar with the fact that his method may be used as a differential staining method for the neuroglia of animals. I find, however, no reference to this fact in his account of his method. This fact seems to me, however, of sufficient importance to warrant my giving his method somewhat in detail, since it does away with one of the obstacles to the use of the Weigert or Mallory method—namely, the necessity of using fresh human tissue in order to study the neuroglia with a differential stain.

The method used by me in my study of the neuroglia of animals is essentially the same as the first of the three methods given by Benda. I have not been successful in staining the neuroglia of animals by the other methods given by him.

The method as used by me is as follows:

1. The tissues, which should be in small pieces, not more than 0.5 cm. in thickness, are fixed and hardened for two to four days in a 4% or 10% solution of formaldehyde (10 parts or 25 parts respectively of formalin in 100 parts of water). A large quantity of the solution is used and, during the hardening process, the tissues rest on several layers of filter paper placed in the bottom of the dish.

2. The tissues are then placed for two to four days in Weigert's chrom-alum mordant, used in his neuroglia stain, the solution being kept in the warm oven at 38° C. during this step.

3. Wash in flowing water for 24 hours.

4. Place tissues for two to four days in a 0.5% aqueous solution of chromic acid.

5. Wash for 24 hours in flowing water.

6. Dehydrate in graded alcohol. It is necessary to dehydrate the tissues very thoroughly.

7. Imbed in paraffin. It is necessary that this procedure be very carefully carried out. After dehydration the tissues are placed in xylol for 24 hours, renewing the xylol several times; then place them for 12 hours in toluol and again 12 hours in benzole. Renew the benzole and add an equal quantity of melted soft paraffin and place in the warm oven. At the end of 24 hours the mixture of benzole and paraffin is replaced by soft paraffin and in three to four hours by hard paraffin (58° C. melting point, Grübler); after three hours' stay in this, they may be imbedded.

8. Cut sections and fix to slide or cover glass with albumin fixative. To facilitate the application of this step, I may say that sections from paraffin-imbedded tissues of the central nervous system are most readily made by placing the knife at an angle of about 30° and placing a layer of distilled water on the knife, renewing it constantly as necessity requires. I have found no difficulty in cutting 3μ to 5μ sections of the spinal cord or even of the medulla of animals ordinarily used in the laboratories. The sections are then caught on a small brush and floated on distilled water contained in a small evaporating dish. When 40 to 50 sections have been cut and floated on the distilled water, the evaporating dish is placed over a flame and the water is gently heated until the sections flatten out, care being taken not to melt the paraffin. The sections are then caught on cover glasses smeared with a thin layer of albumin fixative and placed for 24 hours in the warm oven.

9. Remove paraffin and bring sections through alcohol into distilled water.

10. The sections are now placed for 24 hours in a mordant consisting either of a 4% solution of ferric alum or of a solution of liquor ferri tersulphatis, made by adding one part of this to two parts of distilled water.

11. Sections are rinsed in two tap waters and one distilled water and placed for 24 hours in a solution of sodium sulphalizarate, made by adding to distilled water a sufficient quantity of a saturated solution of sodium sulphalizarate in 70% alcohol to give the distilled water a sulphur-yellow color.

12. Rinse the sections in distilled water and dry between filter papers.

13. Sections are now stained for 15 minutes or longer in a 0.1% solution of toluidin blue, which should be heated, after the sections are in the stain, until the solution steams. Allow the stain to cool and rinse sections in distilled water.

The sections are next rinsed in a slightly-acidulated solution. For this purpose Benda recommends primarily a 1% aqueous solution of glacial acetic acid, in which the sections remain for five to ten seconds and are then dried between filter papers, hastily washed in absolute alcohol and placed in creosote. This step was found necessary in staining the neuroglia of the frog, tortoise and dove. Benda further recommends the use of acidulated alcohol, made by me by adding six drops of hydrochloric acid to 100 ccm. of 70% alcohol. This was found more useful in differentiating the neuroglia of mammalia (dog, cat, rabbit), and experience showed that the proper degree of washing was usually

attained by dipping the sections into this solution as many times as there were micra in the thickness of the section. Tne further procedure is as above.

15. The sections are differentiated in creosote. Benda states that the sections are properly differentiated after they have remained in the creosote about ten minutes. In my work, however, the time varied from about ten minutes to several hours. Sections washed in acidulated alcohol are usually differentiated in about ten minutes; sections washed in the dilute acetic acid solution require a much longer time, varying from one to several hours. It is therefore necessary at all times to control the differentiation under the microscope.

16. After differentiation in the creosote the sections are dried between filter papers, washed in several xylols and mounted in xylol-balsam.

To the naked eye, preparations well differentiated should have a bluish-red or brownish-red color, large masses of neuroglia showing as blue areas. Under the microscope, the neuroglia fibers appear stained deeply blue and stand out very distinctly. The chromatin of the neuroglia cell nuclei presents a purplish-blue color; the remainder of the nucleus is brownish-red and the protoplasm is of the same color, but of a lighter hue. The myelin and neuraxes of the nerve fibers are of a brick-red or brownish-red color, the neuraxes staining much more deeply than the myelin. The nerve cells are of a brownish-red color, the chromophile substance staining more deeply, and the nucleoli are a deep purplish-blue. The fibrous connective tissue stains a pink-red, its nuclei a purplish-blue. The red-blood cells are of a dark greenish-blue. The colors here given are those seen in a well-differentiated preparation, especially after washing in acidulated alcohol (step 14). In case the acetic acid wash is used, the myelin and neuraxes retain some of the blue color, giving them a reddish-purple or even a bluish tinge, the axis-cylinders staining more deeply; even in such preparations, however, the neuroglia fibers may be clearly made out by reason of their deep blue color. It is necessary to add that it is not always possible to obtain a clear differentiation, as the method while often reliable, is not without its whims. It is my custom to fix as many sections to one cover glass as I can, and nearly always some of the sections, if not all, will show the desired result. In over-differentiated preparations, the neuroglia fibers have a brownish-red color, but may usually be clearly seen. Such preparations may, after removal of the creosote and xylol, be brought into water and then stained again in toluidin blue, the further treatment being as above described.

For this investigation material was obtained from the following vertebrates:

Mammalia—Dog, cat, rabbit.

Birds—Dove.

Reptilia—Tortoise (Emys meleagris).

Amphibia—Frog (Rana Catesbiana and Rana halecina).

It is my purpose at this time to give a brief descriptive statement of observations made on the neuroglia of the spinal cord of the above-mentioned vertebrates, differentially stained after Benda's method; I shall deal only with the structure of the neuroglia tissue of adult animals and not with its origin nor its distribution. A much more extended treatment of this subject is contemplated and many of the statements here made will then be substantiated by figures. This will appear on the completion of work now in progress.

Dog. In the dog, the neuroglia of the spinal cord consists of neuroglia cells and neuroglia fibers. With the method used, the nuclei of the neuroglia cells stain a purplish-blue color the protoplasm a brownish-red and the neuroglia fibers a deep blue. The nuclei of the neuroglia cells vary greatly in shape and structure, but may be described under two general types with transition forms. The majority of the neuroglia cell nuclei are vesicular with the chromatin arranged in fine granules. The nuclei belonging to this type vary greatly in shape and size. In the smaller varieties, which are generally of round or oval shape, the chromatin is in the form of numerous small granules; in the larger varieties, which are round, oval, or polymorphous, the chromatin is found in the form of one or several granules, the nucleus being otherwise homogeneous in appearance. The nuclei of the other type, which are not numerous, stain diffusely and usually quite deeply and are usually of round or oval shape and generally quite small. The Benda method has the advantage of staining the protoplasm of the neuroglia cells. The amount of protoplasm seen in connection with the different neuroglia nuclei varies greatly. In connection with the small vesicular nuclei with numerous chromatin granules, it is often difficult to make out any protoplasm, many of them appearing as free nuclei; now and then, however, a thin layer of protoplasm is made out, either surrounding the whole nucleus or appearing at only one side of it. The large vesicular nuclei with one or several larger chromatin granules are usually associated with larger masses of protoplasm, in which case distinct neuroglia cells are distinguished. In cross sections of the spinal cord, such cells present a variety of appearances, their shape depending more or less on the space occupied; they may be triangular or quadran-

gular or somewhat spindle-shaped, with a varying number of processes (usually not more than four or five), which may be traced for short distances between the nerve fibers; such processes are, however, not always made out and when present give the cell a very characteristic appearance. These cells are easily recognized in longitudinal sections of the spinal cord by reason of the structure of their nuclei. The cell protoplasm is often clearly brought out, the cells presenting a rectangular or irregularly oval shape with now and then a few short processes. The small, round or oval deeply-staining nuclei are often associated with a small amount of protoplasm, which usually stains somewhat deeply, often making it difficult to distinguish between protoplasm and nucleus.

The neuroglia fibers stain deeply blue. They vary somewhat in size, but the majority are relatively fine and only here and there does one find coarser fibers. The relation of the neuroglia fibers to the neuroglia cells or free nuclei is generally easily ascertained, in both cross and longitudinal sections of the cord. In the majority of instances, the neuroglia fibers are seen passing over or under the neuroglia cells or nuclei and appear independent of them. Other fibers are seen, however, in close proximity to cells and are seen to lie against them. The large neuroglia cells with protoplasmic branches are particularly interesting in this connection. In such cells it is often possible to trace one or several neuroglia fibers along the side of a protoplasmic branch to the cell body and then either directly across the cell and away from it, or along the side of some other protoplasmic branch and then away from the cell. Three, four or more neuroglia fibers may thus be traced over or under or along the borders of one of these branched neuroglia cells. Usually also numerous small blue dots, cross sections of neuroglia fibers, are seen in close proximity to such cells and now and then in the peripheral portion of their protoplasm; this latter fact must be interpreted as showing that some of the neuroglia fibers pass through the protoplasm of the neuroglia cells. Longitudinal sections of the cord give a better idea of the course of the neuroglia fibers than can be gained from cross sections. In longitudinal sections it will be seen that the neuroglia fibers run parallel to the nerve fibers, at right angles to them and obliquely across them. The relation of the neuroglia fibers to the deeply-staining cells above mentioned is often more difficult to make out, since the cell protoplasm of such cells and the neuroglia fibers now and then stain more nearly the same color than is the case with other cells. In such cells, the neuroglia fibers often *appear* as processes of the cells, especially when a cross section is studied

and when a section is not completely differentiated. In well-differentiated sections and more easily in longitudinal sections, it may usually be made out very clearly that the neuroglia fibers pass over or under or in close proximity to such cells and are not interrupted by them.

Cat.—The neuroglia of the spinal cord of the cat presents essentially the same appearances as those described for the dog. Nearly all of the neuroglia cell nuclei are of the vesicular variety with very little chromatin. In many of the cells, very little protoplasm can be made out, the nuclei appearing as free nuclei. In others, the protoplasm, staining a brownish red color, is readily made out, such cells often appearing distinctly branched, with three, four or five protoplasmic branches, varying in thickness and length and recognized between the cross-cut nerve fibers. The neuroglia fibers of the spinal cord of the cat are somewhat coarser than those found in the dog. They are clearly differentiated and may be traced over or under or around the neuroglia cell nuclei or neuroglia cells. The statements made concerning the relation of the neuroglia cells and nuclei and neuroglia fibers, as seen in the dog, are equally applicable for the cat and need, therefore, no further repetition.

Rabbit.—The neurogliar tissue of the spinal cord of the rabbit is not so easily stained differentially as that of the dog and cat. This is due to the fact that the protoplasm of many of the neuroglia cells shows a greater affinity for the toluidin blue than seems to be the case in the dog and cat and, as a consequence, one often finds neuroglia cells with protoplasm and nucleus staining a purplish color, sometimes of a lighter and sometimes of a darker shade. A longer differentiation in the creosote bleaches not only such cells but also to some extent the neuroglia fibers. The right degree of differentiation is therefore somewhat difficult to obtain. The majority of the nuclei of the neuroglia cells are of the vesicular type, varying greatly in size and shape and in the character and amount of chromatin contained. This may be present in the form of numerous fine granules or as one or several larger granules. Small nuclei, staining deeply, are found, but are not numerous. Many of the nuclei appear as free nuclei and show no or very little protoplasm surrounding them. The large vesicular nuclei, with one or several granules, are generally found in masses of protoplasm which are readily made out. Such cells are usually branched and when stained are purplish-blue in color; the protoplasmic branches can be very readily traced between the nerve fibers. The neuroglia fibers of the rabbit are relatively fine and stain a deep blue color and, in the great majority of cases, it can be readily seen that the fibers are independent of the nu-

clei and protoplasm of the neuroglia cells. In case of the branched neuroglia cells, when the protoplasm is stained a purplish-blue, it is now and then difficult and in certain cells quite impossible, in cross sections of the cord, to differentiate between neuroglia fibers and protoplasmic branches of neuroglia cells. It is often possible to trace with the utmost clearness a neuroglia fiber along the side of a protoplasmic branch of a neuroglia cell, along the side of or over the cell and by the side of some other protoplasmic branch and away from the cell. At other times, however, when a neuroglia fiber appears cut near the cell body of a neuroglia cell or near one of its protoplasmic branches, it appears as if the neuroglia fiber terminated in the cell. In longitudinal sections of the spinal cord of the rabbit, the relation of the neuroglia fibers to the cells under discussion is more readily made out. In such sections, the neuroglia fibers can be readily traced over, under, and around the neuroglia cells, even though at times the color of the neuroglia fibers is similar to that of the protoplasm of the cells.

Dove.—I have had more difficulty in staining the neuroglia of the spinal cord of birds than was experienced with the other vertebrates studied. This is due to the fact that with the method used the neuroglia fibers seem to bleach out at about the same time as the neuroglia cells and nerve fibers. When well stained, the color of the neuroglia fibers is a light blue and, even in the most successful preparations, they do not stand out as clearly as in the mammalia studied. In cross sections of the cord, the great majority of the neuroglia cells of the white matter appear as branched cells with relatively large vesicular nuclei, containing numerous chromatin granules. The protoplasm of such cells often stains a reddish-blue or purplish-blue color. Here and there in the white matter and more generally in the grey matter, free neuroglia cell nuclei are seen of vesicular structure and containing numerous chromatin granules, or similar nuclei surrounded by very little protoplasm.

The neuroglia fibers in the spinal cord of the dove are relatively fine. In longitudinal sections, it can be seen that they are independent of the cell protoplasm of the neuroglia cells; in cross sections of the cord, it is more difficult to see this, but in favorable sections, one can usually trace the neuroglia fibers over or along the borders of the neuroglia cells and gain the conviction that they are not the processes of these cells.

Tortoise.—The neurogliar tissue of the spinal cord of reptilia is quite easily stained by the Benda method, the neuroglia nuclei and cells staining a brownish-red and the neuroglia fibers a deep blue. The nuclei of

the neuroglia cells are generally vesicular and show usually only one round or oval refractive granule, which stains deeply and of a purple color. The amount of protoplasm found with these nuclei varies and oftentimes is scarcely made out. Protoplasmic branches, when present, are slender and short. Here and there are found nuclei, which stain reddish-blue with protoplasm of the same color; such cells usually present clearly-marked protoplasmic branches. The neuroglia fibers vary much in thickness, stain a deep blue and are readily differentiated from the protoplasm of the neuroglia cells and may be traced over and along the borders of the neuroglia cells, often following the protoplasmic branches when these are present.

Frog.—In the frog, the greater proportion of the nuclei of the neuroglia cells are large, round, oval, or polymorphous and vesicular in structure. They are often stained a purplish-blue color, in which case they present a homogeneous appearance; when more bleached, they show numerous small chromatin granules. Here and there, smaller nuclei, staining deeply are also found and are more clearly seen in longitudinal sections. Many free nuclei or nuclei with very little protoplasm are found. The amount of protoplasm found varies greatly with the different cells. The protoplasm usually stains a reddish-blue or purplish-blue color. Branched neuroglia cells are found in the white matter of the cord.

The neuroglia fibers of the spinal cord of the frog are relatively very large and are stained a deep blue color. The greater proportion of these fibers run at right angles to the nerve fibers and in cross sections may often be traced for relatively long distances. In cross sections, not many fibers—usually not more than three or four—are seen in relation with any one neuroglia cell or nucleus. The relation of the neuroglia fibers to free nuclei and neuroglia cells is generally very clearly made out; the neuroglia fibers being so large and of such definite course, we are enabled in both longitudinal and cross sections to differentiate between these fibers and other structures. The neuroglia fibers can usually be traced over or by the side of neuroglia cell nuclei or neuroglia cells and are in no way interrupted by the protoplasm of such cells.

Conclusions.

These observations seem to warrant the following conclusions:

1. The neuroglia of the spinal cord of the dog, cat, rabbit, dove, tortoise and frog consists of neuroglia fibers and neuroglia cells. The neuroglia fibers differ chemically from the protoplasm of the neuroglia

cells—as shown by differential staining—but this difference is not equally well marked in all the forms studied. In the animals studied, this chemical difference between the protoplasm of neuroglia cells and neuroglia fibers is most marked in the dog, cat and tortoise, less so in the rabbit and frog and least in the dove.

II. The neuroglia fibers may be regarded as intercellular structures, as they bear no constant relation to the great majority of the cell nuclei or neuroglia cells observed.

III. By reason of the fact that the protoplasm of the neuroglia cells is stained by the Benda method, this method has been helpful in showing that there are certain neuroglia cells, usually possessing protoplasmic branches, the neuroglia fibers of which are not completely separated from the protoplasm, but are in continuity with it or even pass through it. That such neuroglia fibers are not simply processes of the cells is usually clearly shown by their chemical reaction—behavior toward stains—and by the fact that such fibers may generally be traced over, under, or along the sides of such cells without suffering interruption. That such cells are normal constituents of neurogliar tissue is shown by the fact that they occur in the spinal cord of the four classes of vertebrates studied.

IV. These observations present no evidence which would go to confirm v. Kölliker's hypothesis concerning the structure of neuroglia cells —namely a nucleated cell body with differentiated cell-plate, from which arise processes, the neuroglia fibers. Cross and longitudinal sections of the spinal cord of the animals studied show no such relation of neuroglia fibers and neuroglia cells. These observations cannot be used in confirmation of Reinke's views of the structure of the neuroglia. The branched neuroglia cells seen by me bear no resemblance to the astrocytes or astroblasts, as seen in the chrome-silver preparations with which I am familiar, nor do they resemble the figures given by Reinke. In my own preparations, the neuroglia fibers very generally follow the course of the protoplasmic branches of the neuroglia cells.

V. The observations here presented strengthen materially the position held by Weigert and Mallory as to the structure of neuroglia tissue, as well as by others who may have used their methods or modifications thereof in the study of neuroglia, and extend their observations to cover the more important vertebrate classes.

Finally the following quotation from Pollack's article may serve to emphasize the further advantages of the method used by me: "Angesichts des Umstandes aber, dass wir des Thierexperimentes nicht entrathen können, da wir ja am Menschen keine Versuche mit experi-

menteller Degeneration anstellen können, erscheint mir die Anwendbarkeit auf das thierische Nervensystem als das nächste Postulate dieser hochbedeutsamen Methode." This postulate, it seems to me, has to a large extent been met by the introduction of the Benda differential neuroglia stain. I may add that experimental work on cats and dogs, undertaken with a view of studying the behavior of the neuroglia under certain pathologic conditions has been begun and will form the subject of some future contribution.

In conclusion I wish to thank Mr. August Henry Roth, student in medicine, for much valuable assistance given in the preparation of the sections on which these observations are founded.

REFERENCES TO LITERATURE.

1900. J. A. Aguerre.—Untersuchungen über die menschliche Neuroglia. Archiv f. Mik. Anatom. und Entwicklungsgesch., Vol. LVI, p. 509.

1893. W. Lloyd Andriezen.—The Neuroglia Elements in the Human Brain. Brit. Med. Journ., Vol. II, July-Dec., p. 227.

1900. C. Benda.—Erfahrungen über Neurogliafärbungen und eine neue Färbungsmethode. Neurologisches Centralblatt, Vol. XIX, p. 786.

1901. A. Bonome.—Bau und Histogenese des pathologischen Neuroglia-Gewebes. Virchow's Archiv, Vol. CLXIII, p. 441.

1897. F. W. Eurich.—Studies on the Neuroglia. Brain, Vol. XX, p. 114.

1898. F. W. Eurich.—Contribution to the Comparative Anatomy of the Neuroglia. Journal of Anatomy and Physiology, Vol. XXXII, p. 688.

1899. R. Krause.—Untersuchungen über die Neuroglia des Affen. Anhang z. d. Abh. der Königl. Akad. der Wissenschaften zu Berlin. 1899.

1900. R. Krause and J. Aguerre.—Untersuchungen über den Bau des menschlichen Rückenmarkes mit besonderer Berücksichtigung der Neuroglia. Anat. Anzeiger, Vol. XVIII, p. 239.

1893. A. v. Kölliker.—Handbuch der Gewebelehre des Menschen, 6 Aufl., Vol. II, pp. 136 to 193.

1895. F. B. Mallory.—Centralblatt f. Allg. Pathol. u. path. Anat., Vol. VI, p. 753; also Method of Fixation for Neuroglia Fibers, pp. 532-3, Journal of Exp. Med., Vol. II, 1897.

1899. Erik Müller.—Studien über Neuroglia. Arch. f. Mik. Anatom. und Entwicklungsgesch., Vol. LV, p. 11.

1897. B. Pollack.—Einige Bemerkungen über die Neuroglia und Neurogliafärbung. Arch. f. Mik. Anatom. und Entwicklungsgesch., Vol. XLVIII, p. 274.

1897. Fr. Reinke.—Ueber die Neuroglia in der weissen Substanz des Rückensmarks vom erwachsenen Menschen. Arch. f. Mik. Anatom. und Entwicklungsgesch., Vol. L, p. 1.

1899. E. Storch.—Ueber die pathologish-anatomischen Vorgänge am Stützgerüst des Centralnervensystems. Virchow's Archiv, Vol. CLVII, p. 127.

1897. E. W. Taylor.—A Contribution to the Study of Human Neuroglia. The Journal of Experimental Medicine, Vol. II, p. 611.

1895. C. Weigert.—Beiträge zur Kenntniss der normalen menschlichen Neuroglia. Festschrift, Frankfurt a M.

1898. J. R. Whitwell.—On the Structure of the Neuroglia. British Med. Journ., Vol. I, Jan.-June, p. 681.

1900. K. Yamagiwa.—Eine neue Färbung der Neuroglia. Virchow's Archiv, Vol. CLX, p. 358.

THE NORMAL HISTOLOGY OF THE HUMAN HEMOLYMPH GLANDS.

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In the prevertebral fat of the bullock and sheep there are found in large numbers glands varying in size from a pin-point to a large cherry and of a deep-red or chocolate color. Because of their number and striking color these glands stand out with much greater prominence than the pale lymph glands of these animals, but despite this fact they have been strangely overlooked and their histology and function investigated by but few observers. The earliest mention made of them that I have been able to find is by Leydig (Lehrbuch der Histologie der Menschen und der Thiere, 1857, pages 424, 429). This writer speaks of glands occurring along the thoracic aorta in many mammals, particularly in the hog, which from their color might be mistaken for accessory spleens in case they were found in the immediate neighborhood of the spleen. The cut surface of many of these glands he describes as perfectly resembling that of the spleen, presenting a dark-red pulp in which lie white masses of cells corresponding to the Malpighian follicles. Leydig noticed further that transition-forms exist between these glands and ordinary lymph-glands, but his observations were confined entirely to the gross appearances, and the histological structure of these glands was not investigated by him.

In 1884 H. Gibbes noticed the presence near the renal vessels of the human body of glands differing from ordinary lymph-glands in that they possessed sinuses containing blood in place of lymph. This important discovery was not carried further and the observation was lost sight of, the next mention of these organs being made by Robertson (Lancet, Nov. 29, 1890) who, apparently unaware of Gibbes' discovery, made the first histological study of these structures and gave them the name of hemolymph glands (suggested by Dr. Russell under whom Robertson was working). He found these glands to be present in the sheep, bullock and human body, but his histological descriptions are based chiefly upon their structure as found in the sheep. His work

upon the human body was unsatisfactory because of the unfavorable conditions under which his autopsies were made. He was able, however, to determine the presence of these glands in man, and states that their structure in the human body is very similar to that in the sheep and bullock.

In 1891 Clarkson reported observations on "certain hitherto undescribed glands" found in the neighborhood of the renal vessels in the horse, sheep and goat. To these he gave the name of "hemal glands," and considered them to be a different variety from the hemolymph glands described by Robertson though probably possessing the same function.

Gibbes in 1893 made a second report in which he states that Robertson's description of these organs accords with the glands discovered by himself in 1884, and accepts the designation of hemolymph glands as an appropriate name for them. Finding his discovery thus confirmed he had made further investigations in the human subject, and found these glands to be constantly present near the renal vessels. He added nothing, however, concerning their histology or function.

In Clarkson's "Text-Book of Histology," 1896, several pages are devoted to the histology of the "hemal glands." Clarkson states that these organs have been found in the pig, horse, ox and sheep, but have not yet been observed in man. His descriptions are similar to those of Gibbes and Robertson, and the hemal glands must be regarded as hemolymph glands.

Vincent and Harrison, in 1897, gave a detailed histological description of the hemolymph glands of the ox, sheep and rat. They noted also the occurrence of similar glands in the horse, dog, common fowl and turkey, and pointed out the histological resemblance existing between hemolymph glands and the head-kidney of certain teleostean fishes. In several human cadavers examined they found no hemolymph glands, but Vincent in a later examination found them in the mesentery of a young boy.

In 1900 Drummond made a more thorough study of the histology of the hemolymph glands of the sheep, ox, rat and dog; and gave a very clear description of their structure. He confirmed the work of Robertson and Vincent and Harrison; and added many important points concerning the distribution and structure of these glands in the lower animals.

In so far as I have been able to discover these are the only observations that have been made of the occurrence and structure of the hemolymph glands, and as seen above the chief part of these have been

of these glands as occurring in the lower vertebrates. Gibbes' discovery of their presence in the human subject, confirmed by Robertson, the second report of the former and the solitary observation of Vincent represent the sum total of the published work upon the human hemolymph glands up to the publication of my article, "A Contribution to the Normal Histology and Pathology of the Hemolymph Glands" (Journal of the Bost. Soc. of Med. Sciences, April, 1901). In this paper I made a preliminary report of a study of the human hemolymph glands which has been carried on by me in this laboratory for several years. My attention was first drawn to these glands by certain cases in which some of the retroperitoneal lymph glands appeared to play a part independent of that of the other lymph glands of the body. In its earlier stages the study was confined to the retroperitoneal region, but later systematic investigation was also carried out of the cervical, thoracic and mediastinal glands. The material for this work has been obtained from my autopsy cases of the last five years, 94 in all. It was early evident to me that there were two distinct varieties of lymph glands, one containing blood sinuses and apparently possessing distinct hemal functions. At that time the observations of Gibbes, Robertson and Vincent were unknown to me so that the essential nature of the glands was also independently discovered by me.

Since the publication of my article in April of this year there has appeared in the Archiv f. Mikr. Anatomie (July) a paper by Weidenreich on the "Gefässsystem der Menschlichen Milz," in which he treats theoretically of the hemolymph glands, basing his conclusions upon the work of the English observers. The more important of his theoretical deductions are confirmed by my previously-reported observations.

The present paper will be devoted to a consideration of the normal histology of human hemolymph glands to a much fuller extent than in my preliminary report. With the exception of a few accident cases none of my autopsy subjects can be said to have been normal. The majority were chronic cases, many of which showed various stages of anemia and cachexia. In so far as I am able to divide the appearances observed by me into histological and pathological such classification is based upon the fact that only in cases showing extensive changes in the blood were conditions found in the hemolymph glands that could be regarded as being essentially pathological. In all other cases the structure of the glands was assumed to be histological because of their identity of structure with the glands found in normal individuals killed by accident, the general similarity of structure in all cases except certain blood cases, and finally their resemblance to the hemolymph glands of

normal animals. As a result of this study the following conclusions regarding the normal histology of the human hemolymph glands have been reached.

Technique.—The thymus, anterior mediastinal and renal regions are examined in the usual order of the autopsy; the prevertebral tissues at the close of the autopsy. The neck-organs, thoracic and abdominal vessels, root of the mesentery, and other structures attached are stripped from the spinal column from above downwards and removed from the body for minute examination. When there is much prevertebral fat present or when the tissues are very opaque the search for hemolymph glands may be made much more successful by first fixing the tissues in mass in 4 per cent formalin. The color of the blood-sinuses is in this way brought out much more sharply, and the glands may be recognized when the search in the fresh tissue would have been negative. When it is desired to study the exact position and relations of these glands the prevertebral tissues should be dissected in place and not stripped from the spine. Since the sinuses of the human hemolymph glands partly collapse after death the recognition by color alone is not easy and at times impossible. It therefore becomes necessary to remove all glands found in a region for microscopical diagnosis and in many cases it is only in this way that the character of a gland can be definitely determined. In the search for these glands in the human body their proximity to large vessels should be borne in mind; they occur very frequently in the connective tissue between artery and vein.

Occurrence.—Taking the presence of a sinus containing blood in place of lymph as the essential feature of a hemolymph gland, such glands are found constantly present in the human body. They are apparently more numerous, at least are more easily recognized, in early adult and middle life than in infancy or old age. In new-born children they are discovered with difficulty, and usually can be found only after microscopical examination. In late life they become atrophic, the blood-sinuses being obliterated for the greater part by connective-tissue increase. No difference in their occurrence has been observed in the sexes. With the individual they apparently differ very much as regards their location, number and size, seldom being found under exactly similar conditions; but owing to the difficulty of recognizing them with the naked eye it is probable that these variations may be only apparent, resulting from imperfect technique.

They are found in greatest numbers in the prevertebral retroperitoneal and cervical regions, in the neighborhood of the adrenal and renal vessels, along the brim of the pelvis, in the root of the mesen-

tery but rarely extending far out into it, still more rarely in the omentum and epiploica. In normal individuals they are rarely found in the mediastinal tissues or along the thoracic vertebrae, but in cases of anemia in which the hemolymph glands throughout the entire body are enlarged they may be found in large numbers in these regions making it appear probable that under normal conditions they are of such small size as to escape notice. In the cervical region they are usually found below and behind the lobes of the thyroid in association with the parathyroids.

Gross Appearances.—The human hemolymph glands are not nearly so easily recognized by the naked eye as are those of the steer and sheep, because of the fact that their blood-sinuses are frequently collapsed and partly emptied after death. They usually lie deeply embedded in fat or connective tissue, and as a rule near the wall of some large vessel. As a rule only a few show distended sinuses, these glands are deep-red or bluish in color and may be easily mistaken for hemorrhages, blood-clots, or deeply-congested lymph glands. The resemblance to spleen-tissue is often very close. In the transition forms which are partly hemolymph glands and partly ordinary lymphatic glands the blood-sinuses appear as red points or streaks. It is often difficult or impossible to distinguish these from congested lymph glands. The smallest hemolymph glands may often be found by stretching the tissue against the light, the blood-sinuses then appearing as red points or lines. Fixation of the tissues in formalin is of great advantage as the blood-content of the sinuses of these glands is brought out in sharp contrast to the lighter color of lymphoid tissue. When the blood-sinuses are small or few in number the gland cannot be distinguished on naked-eye inspection from an ordinary lymph gland. It is, therefore, safest in studying the occurrence of hemolymph glands to remove all apparent glandular structures and examine them microscopically.

On cross-section the blood-sinuses resemble spleen-pulp and contrast according to their blood-content more or less sharply with the whitish areas of lymphoid tissue. Small round masses of whitish lymphoid tissue often project into the pulp-like peripheral sinus suggesting splenic follicles. Partly collapsed and emptied sinuses appear as points and streaks of red. The presence of a peripheral red streak just beneath the capsule of the gland with red lines radiating toward the center of the gland is a very important point in the naked-eye diagnosis of these organs. Occasionally the capsular surface of a hemolymph gland is studded with small beaded elevations, giving it a raspberry appear-

ance; on section these are found to be small round masses of lymphoid tissue projecting into the peripheral sinus.

The size of the human hemolymph glands varies from that of a pinpoint to a large cherry or almond. The latter size is, however, uncommon, the usual size being that of a yellow mustard seed or pea. In the majority of cases their shape is round or oval, some are flattened, others elongated, while not infrequently glands of an almond shape are found, the larger end being somewhat recurved upon itself. They usually possess a distinct hilum into which vessels enter. Their consistency is somewhat softer than that of ordinary lymph glands depending upon the amount of blood in the sinuses. If these are large and dilated the capsule of the gland may be so stretched that it is easily ruptured and the gland pulp have the appearance and consistency of a fresh blood-clot; and these glands are undoubtedly many times mistaken for such.

Attached to the gland there is usually a relatively large plexus of vessels, the veins in particular being large and prominent. Occasionally these remain dilated and filled with blood, and under such conditions are of great aid in making the naked-eye diagnosis. No lymph vessels can be demonstrated in the case of those glands, even the largest, which contain blood-sinuses throughout, but in the glands of mixed type, partly hemolymph and partly lymphatic, lymph vessels can be made out.

The number of hemolymph glands in the human body is exceedingly difficult of estimation. Since the ultimate diagnosis depends upon the microscopical examination it would be necessary in making an exact estimation to examine every lymphatic gland in the body. Moreover, since many of the hemolymph glands are very small and lie deeply embedded in fat and connective tissue, it is necessary to remove all the tissues in the regions where the glands are found and make serial sections of the entire tissue. The difficulty of this is evident. The number of lymph glands visible to the naked eye in the retroperitoneal region varies from 200-500, and the prevertebral tissues contain also numerous nodes of lymphoid tissue too small to be seen on naked-eye inspection. In a number of cases the entire retroperitoneal tissue has been examined, both in the fresh state and microscopically, but the results are so much at variance that no definite statements regarding the number of hemolymph glands can be made. Ordinarily the relative proportion to lymphatic glands is 1-20 to 1-50, but this statement is based upon very incomplete observations. It is very probable that the number of hemolymph glands is much greater than that expressed by these ratios, since in one case of pernicious anemia over sixty of the glands were removed

from the cervical, thoracic and retroperitoneal regions alone; in another case of pernicious anemia, over thirty from the retroperitoneal region alone; and forty from the same region in a case of leukemia. Vincent's finding of hemolymph glands, about fifty in number, in the mesentery and gastro-colic omentum may also be remembered. In the human body many of these glands appear to be in a resting state, and are not easily distinguished from ordinary lymph glands, but in certain conditions, particularly blood diseases, they become enlarged and prominent. A new-formation of these glands in compensation for spleen or bone-marrow is also possible and undoubtedly takes place under certain pathological conditions. The use of formalin in fixing the tissues and rendering more prominent the blood-content of hemolymph glands may be mentioned again in this place as being of great service in the estimation of their number.

Microscopical Examination.

Technique.—Alcohol fixation does not give the best results in the study of these glands except for the use of the tri-acid stain. It produces too much contraction of the reticulum and changes the red cells so that they do not stain well. Mercuric chloride, formalin and Zenker's fluid are the best fixing agents in the order named. With all of these there is less shrinkage, and the red cells are better preserved, staining well with eosin, etc., so that the course of the blood-sinuses is well outlined by the blood yet remaining in the meshes of the reticulum. Mercuric chloride fixation is especially recommended in the study of their finer structure, since all stains, including the tri-acid, give good results after this fixation, the cell-division figures and the red-blood cells are well preserved, and there is little shrinking. Hematoxylin and eosin, the tri-acid stain, etc., are used according to the object sought. Mallory's reticulum stain is essential for the study of the reticulum of the blood-sinuses, and is particularly valuable in tracing the course of these. Kresylechtviolett is also very valuable in the study of mast-cells, etc.

For the study of the cells of these glands it is also advisable to make cover-glass smears from the freshly-cut surface. This should be done as soon as possible after death. The smears are made in the usual manner, fixed by heat or alcohol and ether, and stained as desired. In this connection kresylechtviolett is also recommended as of value in the study of cell-granulation.

Microscopical Appearances.—The lymph glands of the human body may be divided broadly into two groups: those containing only lymph-sinuses, ordinary lymphatic glands, and those possessing blood-sinuses, hemolymph glands. Between these two groups intermediate forms exist—a gland may contain both blood and lymph-sinuses—the pres-

ence of a blood-sinus, however small, is sufficient warrant for the classification of a gland as a hemolymph gland.

The microscopical study of the lymphoid nodes containing blood-sinuses reveals a most striking variety of structure in so far as the relative size, number and arrangement of the blood-sinuses, lymphoid tissue, etc., are concerned. It is possible, however, to divide the different forms into two distinct types, to which I have given the names *splenolymph* and *marrowlymph* gland as indicating their structure and probable functions. Between these two there is every possible transition-form, just as there is also between the spleen, hemolymph glands and ordinary lymphatic glands. It is not by any means intended to replace the designation, hemolymph gland, by these names, as the latter should still be used as a collective term.

Splenolymph Glands.

The great majority of hemolymph glands correspond to this type. They are found chiefly along the abdominal aorta, vena cava, adrenal and renal vessels, in the neighborhood of the solar plexus, cervical region, occasionally in the omentum, mesentery, epiploica, mediastinal and thoracic prevertebral regions. These glands are usually round, but also frequently almond-shaped, varying in size from a pin-point to a large cherry. As a rule they possess a distinct hilum into which numerous vessels of large size enter. These are also found penetrating the capsule at many points. Very often the gland appears to be surrounded by a plexus of vessels, sometimes arterial, at other times venous. Their gross appearances correspond with those given above for hemolymph glands in general. Their resemblance to the spleen is sometimes so great that they may be mistaken for accessory spleens, and undoubtedly many of the so-called accessory spleens belong to this type of hemolymph gland. This fact was dimly recognized by Haberer in his recent article "Lien Succenturiatus und Lien Accessorius" (Arch. f. Anat. u. Phys., March, 1901). Apparently unaware of the existence of hemolymph glands this observer concluded that many glands regarded as accessory spleens were in reality peculiar types of lymph glands representing intermediate forms between spleen and lymph glands.

Capsule.—The splenolymph glands possess a capsule of connective tissue which may be very thick in proportion to the size of the organ or very thin and delicate. It contains a varying amount of unstriped muscle, and very little yellow elastic tissue. Adipose tissue surrounds the capsule. The latter is frequently pierced by many obliquely-pene-

trating blood-vessels. Occasionally the blood-spaces and vessels in the capsule are so numerous as to give it the cavernous structure so frequently seen in the capsule of these glands in the steer; but on the whole the splenolymph glands of man are distinguished from those of the lower animals by the less vascular structure of the capsule. From the external capsule trabeculæ of similar tissues run into the gland dividing it into irregular lobules. Accompanying the trabeculæ are the communicating blood-sinuses and between them lies the lymphoid tissue.

Blood-sinuses.—Immediately beneath the external capsule there is a blood-sinus which sometimes extends entirely around the periphery of the gland, but more frequently only for portions of the way, being frequently interrupted by masses of lymphoid tissue which reach to the external capsule. In the great majority of cases this sinus is much smaller and less prominent than in the hemolymph glands of the steer and sheep. Glands are, however, occasionally seen in man with the peripheral sinus dilated and containing as much blood as in any gland from these animals. From the peripheral sinus branches pass in with the trabeculæ toward the centre of the gland or toward its hilum, increasing in size and becoming confluent towards these points until they are often very large and prominent. These radiating sinuses frequently communicate with each other, and so divide the lymphoid tissue into irregularly-shaped islands apparently surrounded on all sides by a blood-sinus. Serial sections, however, show that these islands are not entirely cut off from each other in the majority of cases, but at some point or other are connected by an isthmus of lymphoid tissue of varying size. The number and size of the blood-sinuses as well as their general arrangement vary greatly, so that scarcely any two glands exactly resemble each other in these respects.

The lumen of the peripheral as well as of the communicating and central sinuses is traversed by a coarse reticulum through the meshes of which the blood circulates. The amount of this reticulum and the size of its meshes vary in different glands; frequently the central sinuses are open, possessing but a scanty reticulum or none at all. The reticulum of the sinuses is probably lined throughout with flattened endothelium, but it is sometimes difficult or impossible to make this out, so that the blood appears to be in direct contact with fibres of the reticulum. A wide sinus is often abruptly narrowed by a constriction of coarser reticulum suggesting a valve-like arrangement, but this point is yet to be worked out. The course of the sinuses is clearly shown in sections by the lighter staining nuclei of the reticulum in contrast

to the more deeply-stained lymphoid tissue and the red cells lying in the reticular meshes.

The central sinuses frequently form the most striking feature of the splenolymph glands. Though usually partly or even wholly emptied of blood they often remain dilated. Scanty reticulum may extend either across or only for a short distance into the lumen of the larger sinuses. At other times the central sinuses may contain as much reticulum as either the peripheral or communicating ones. Red-blood cells and large phagocytes containing red cells and blood pigment are found in the reticular meshes along the sides of these sinuses. In some glands the central and communicating sinuses take up the greater part of the central portion of the gland, so that microscopical sections resemble a much-congested spleen-pulp.

Lymphoid Tissue.—The lymphoid tissue lying between the sinuses resembles very much that of an ordinary lymph gland. It varies very much in amount, sometimes forming a mere network between the sinuses, while in other cases it may form the chief part of the gland. Usually the greater mass of lymphoid tissue is toward the periphery, forming the inner border of the peripheral sinus, but it also frequently extends to the capsule interrupting the peripheral sinus. The arrangement of the lymphoid tissue also varies much in different glands. It is usually cut up into irregular areas or lobules which are for the greater part surrounded by the blood-sinuses. Small round collections of lymphoid cells are often seen, resembling splenic follicles. These occur more frequently at the periphery, where they may be partly or wholly surrounded by the blood-sinus, but they may also be found toward the central portion of the gland. Serial sections show that they are almost perfectly round. In the majority of cases they possess no arterial relations as in the case of the splenic follicles, but occasionally a small capillary is found in them which under certain pathological conditions may become gradually converted into a small arteriole with thick walls. The resemblance in this case to the splenic follicle is complete.

The cells of the lymphoid tissue are for the greater part small lymphocytes. These vary greatly with respect to the relative size and staining power of the nucleus and relative amount of protoplasm. Next to the small lymphocyte the large mononuclear cell is the most common form present. These also vary much in size, form and staining power. Transitional and polymorphonuclear leukocytes are also present. A small number of basophile and mononuclear eosinophiles is usually present, mast-cells are rare in the majority of cases, but occasionally a

gland is found, as in the steer and sheep, in which the majority of the cells of the central portion of the gland are mast-cells.

Red-blood cells lie free in the meshes of the reticulum. The small blood-vessels and capillaries of the lymphoid tissue are usually filled with red cells and leukocytes, the large mononuclear form appearing to predominate. Throughout the reticulum there is usually present a varying amount of blood pigment, partly free and partly contained within large mononuclear phagocytes. Red cells in various stages of disintegration are also found in these cells. Scattered areas of a hyaline substance which stain pink with eosin, red with fuchsin, and blue with Mallory's reticulum stain are often found throughout the lymphoid tissue, but are most numerous toward the periphery of the gland. Small hyaline, highly refractile spherules of varying size, usually about the diameter of a red-blood cell, are frequently seen in small groups lying free in the reticular meshes and are also found in the mononuclear phagocytes. They stain intensely with eosin and fuchsin, retaining the latter with Mallory's reticulum stain. These hyaline bodies are evidently the products of the destruction of red-blood cells, as all stages of their formation can be found. They may contain iron, especially those found in the phagocytes, the reaction being absent from many of the free bodies. In some cases these spherules can be seen partly extruded from the phagocyte.

Reticulum.—The reticulum of the lymphoid areas resembles that of ordinary lymphatic glands. That of the blood-sinuses is much more abundant and of a coarser mesh-like structure than that of the lymph-sinuses of lymph glands. It differs from the reticulum of the spleen-pulp in the same respects. It stains blue with Mallory's reticulum stain, and consists of branching fibres and stellate or spindle-shaped cells arranged in a coarse mesh-work, the surfaces of which are covered with flattened endothelial cells. Small fibrillæ of yellow elastic tissue and occasional unstriped muscle-cells may be scattered through it. In its meshes there are found constantly large mononuclear phagocytes containing disintegrating red cells. These cells are always more abundant in the reticulum of the sinuses than in the lymphoid tissue. Under certain conditions they are so greatly increased in number as to almost entirely fill the spaces of the sinus. Normally their number in individual glands varies greatly, suggesting a possible cyclical function of hemolysis. Glands containing many of these cells may be found side by side with others whose reticular spaces contain but few. The same appearances are found in the hemolymph glands of the lower animals, particularly in those of the dog and rat. Multinuclear giant-

cells, eosinophile, basophile and mast-cells may at times be found also in the reticular meshes. The origin of the phagocytes has not yet been definitely determined; they may arise either from leukocytes or from the endothelial cells lining the reticulum.

Vascular System.—The exact manner of circulation in these glands has not yet been worked out, but it seems probable that the arteries entering the hilum quickly divide into small branches which, passing toward the periphery, empty into the blood-sinuses from which the blood is again gathered into veins which pass out at the hilum or obliquely through the capsule. As in the lower animals, there are great individual differences in the number and mode of branching of the blood-vessels. Occasionally the entering arteries pass along the trabeculæ and do not divide until near the periphery. In well-stained sections the course of the blood-sinuses and vessels is well shown by the blood contained in them which serves the purpose of an injection. The exact manner of communication between arterial and venous systems cannot, however, be made out by this means. Injections have not yet been attempted in the human subject, but in the lower animals they have been unsatisfactory. The circulation in the sinuses is of the type known as sinusoidal, only the endothelium separating the blood from the cells contained in the reticular meshes. The current in these spaces must be extremely slow and a long period of time must be required for the complete circulation through the intricate meshes of the reticulum crossing the sinuses.

In the splenolymph glands containing blood-sinuses throughout, afferent and efferent lymph vessels are not found, small lymphatics alone being present in the capsule. In glands of mixed type containing lymph-sinuses lymphatic vessels are also found. Whether there is any communication between the lymphatic and blood-systems in these glands remains yet to be shown. In the round masses of cells lymph-spaces or capillaries are probably present as in the splenic follicles, since under certain pathological conditions these may become edematous, the cells being separated by an accumulation of fluid in the intercellular spaces. These lymph spaces probably empty directly into the blood-sinuses and convey leukocytes into the circulation.

Varieties.—As already mentioned transition forms exist between the splenolymph glands and the spleen on one hand and ordinary lymphatic glands on the other. The significance of these intermediate forms is not yet apparent.

Development.—No work has yet been done on the development of these glands. They are present as fully-developed organs in the new-

born child, and have been found at an early period in the fœtal calf. They are without doubt individual organs whose early stages of development are probably parallel with those of the lymph glands. Under certain pathological conditions it is possible that they may be developed from ordinary lymphatic glands or even in adipose tissue in compensation for the spleen or bone-marrow.

Function.—A discussion of the probable functions of these glands is beyond the limits of this paper. Briefly stated, it is probable that their function is chiefly one of hematolysis. The great variation in appearances in different glands suggests a cyclical function. They are also leukocyte-forming organs. Under normal conditions no evidences of red-blood cell formation have been discovered in them. It may also be possible that in the glands containing both blood and lymph-sinuses there is communication between the two systems and these glands may serve as the means of the return of lymph into the blood-stream. This supposition must, however, be regarded as purely hypothetical.

Differential Diagnosis.—To the inexperienced observer the hemolymph glands may at first be taken for deeply-congested or hemorrhagic lymph glands, even on microscopical examination. This mistake has probably occurred many times. The essential features of these glands as given above are, however, easily seen and, when once known, a glance at a section is sufficient for their recognition. The differences in reticulum, lymphoid tissue, sinuses, etc., together present a picture entirely distinct and characteristic from that of a congested or hemorrhagic lymph gland. Care in the treatment of the autopsy material, perfect fixation and good staining are points of technique which are of great service in the recognition of these organs.

MARROWLYMPH GLANDS.

The second form of hemolymph gland to which I have applied the designation of marrowlymph gland is of very much less frequent occurrence. They are much more prominent in certain pathological conditions than they are in the normal body, suggesting the possibility of resting glands or of new formations. In many cadavers I have been unable to find them, but this may have been due to a lack of time for a thorough examination. They have been found only in the retroperitoneal region, along the spine and brim of pelvis, always in close proximity to the large vessels, vena cava, abdominal aorta, adrenal and renal vessels and common iliacs. They are present most frequently

along the vertebræ behind the aorta or between it and the vena cava. These glands are flattened and long in proportion to their breadth, their greatest dimension lying parallel to the axis of the neighboring vessel. They may have a distinct hilum, but the number of vessels entering is not so great as in the case of the splenolymph glands. Lymph vessels may also be found in connection with these glands. The latter vary greatly in size, but are sometimes found as slender cylinders several centimeters long embedded in adipose tissue. They are white or pinkish in color with fine red lines corresponding to the blood-sinuses. Their consistence is very soft and on section they present an almost homogeneous surface.

They possess a thin capsule which contains but little unstriped muscle and yellow elastic tissue. Delicate trabeculæ run from this toward the centre of the gland. Beneath the capsule there is a blood-sinus of small size which usually runs entirely around the periphery and from this there are narrow branching sinuses accompanying the trabeculæ toward the centre of the gland. All of the sinuses are filled with a coarse reticulum through the meshes of which the blood circulates. Dilated sinuses like those of the splenolymph glands are not present. The course of the sinuses is shown by the lighter staining nuclei of the reticulum and by the presence of red-cells. Lymph sinuses are also present in some of these glands. Between the sinuses lies the lymphoid tissue arranged in irregular islands or lobules and is in much greater amount than in the splenolymph glands. Collections of cells resembling follicles are not found in the pure type of this gland. Throughout their central portions large numbers of fat cells are usually present.

The reticulum of the lymphoid tissue is more delicate and contains but little elastic tissue. The lymphoid areas are richer in cells and these present a much greater variety than in the splenolymph glands. Mononuclear eosinophiles and mast-cells are more numerous, and multinuclear as well as large mononuclear forms with deeply-stained knobbed nuclei also occur. Giant-cells resembling those of the bone-marrow are also occasionally found, and in certain pathological states of the blood may be very numerous. No nucleated red cells have been found in normal conditions. The lymphocytes and large mononuclears show a much greater diversity of form and staining power, and there is also a great variety of cells in the reticulum of the sinuses. Phagocytes containing red-cells pigment, fuchsinophile hyaline bodies and leukocytes occur to a much less extent than in the splenolymph glands. Red-

blood cells are found scattered throughout the reticulum of the lymphoid tissue.

Transition-forms between spleno- and marrowlymph glands are found and also between the latter and ordinary lymphatic glands. The function of the marrowlymph glands is not clear. They are evidently leukocyte-forming organs, and the presence of giant-cells and many mononuclear eosinophiles suggests the bone-marrow and the formation of red-cells. In certain forms of anemia and leukemia these glands are greatly enlarged and come to resemble the lymphoid marrow, containing nucleated red cells, neutrophile and eosinophile mononuclears, and many giant-cells exactly similar to those of the marrow. It can hardly be doubted that in these cases these glands are centres of red-cell formation perhaps compensatory for the marrow. Under normal conditions they may be in a resting state in so far as their function is concerned. Their part in hemolysis is much less marked than that of the splenolymph glands. In old age they become atrophic, their sinuses obliterated, large deposits of hyalin occur in them, and finally they cannot be distinguished from atrophic lymphatic glands. The study of these glands from the pathological side promises much more than from the normal.

SUMMARY.

Our conceptions of lymphoid tissue are greatly broadened by the study of the hemolymph glands. The intimate relations existing between the different organs of this class, the transition-forms, the possibility of compensation of function, etc., throw new and important light upon both histological and pathological problems. The various lymphoid organs might be compared as follows:

1. LYMPHOID MARROW:
   - Hemal gland.
   - Blood-forming function.
   - Sinusoidal organ.
   - Afferent and efferent blood-vessels.
   - No afferent lymph-vessels.

2. HEMOLYMPH GLANDS:
   - Hemolymph gland.
   - Hemolytic function.
   - Sinusoidal organ.
   - Afferent and efferent blood-vessels.
   - No afferent lymph-vessels.
   - Efferent lymph-capillaries.

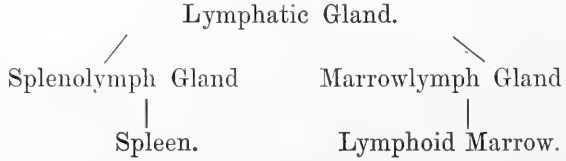
3. SPLEEN:

Hemolymph gland.
Hemolytic function.
Sinusoidal organ.
Afferent and efferent blood-vessels.
No afferent lymph-vessels.
Efferent lymph-capillaries.

4. LYMPHATIC GLAND:

Lymph gland.
Lymphatic function.
Lymph-sinuses.
No blood-sinuses.
Lymph and blood-systems separate.

With respect to the relations between blood and lymphatic systems the red marrow might be considered the most primitive type of lymphoid structure, and the ordinary lymphatic gland the most highly developed, the hemolymph glands and spleen occupying intermediate positions. If viewed from a broader standpoint regarding both general structure and functions the relations of the organs might be theoretically represented in the following manner:



In conclusion, the field of the human hemolymph glands has barely been entered and its most important problems remain unsolved. Much is to be hoped from experimental work upon the lower animals in whom these glands are larger and more numerous than in man. Work along this line has already been begun in this laboratory.

NOTE.—The limits of this article have prevented me including in the brief review of the literature given above references to observations made on *lymph-glands with blood-containing sinuses* by a number of writers who apparently unaware of the existence of hemolymph glands interpreted their findings as hyperemic or hemorrhagic lymph-glands. Rindfleisch, Weigert, Neumann and Orth are among those who have made such observations. Especial attention is, however called to the article by Saltykow (Ueber bluthältige Lymphdrüsen beim Menschen, Zeitschr. f. Heilkunde, 1900). In an examination of 60 cadavers this

observer found blood-containing lymph-glands in 91.66% of the cases. Saltykow considered these glands to be only ordinary lymphatic glands in which there had been hemorrhage or a backward flow of blood into the lymph-vessels. Referring to the hemolymph glands as described by Gibbes, Robertson and Clarkson he concludes that these writers were in error in their assumption that these were glands sui generis. I have already pointed out the close resemblance between hemorrhagic lymph glands and hemolymph glands and some of the more important differential points between these structures. I regard Saltykow as being wholly in error in so far as his conclusions regarding the nature of these glands are concerned.

BIBLIOGRAPHY.

Clarkson.—British Med. Jour., July 25, 1891.
——— Text-book of Histology, 1896.
Drummond.—Jour. of Anat. and Phys., 1900.
Gibbes.—Microscopical Journal, Vol. XXIV, 1884.
——— Amer. Jour. of Med. Sciences, 1893.
Haberer.—Arch. f. Anat. u. Phys., March, 1901.
Leydig.—Lehrbuch der Histologie d. Menschen u. d. Thiere, 1857.
Robertson.—Lancet, 1890.
Vincent and Harrison.—Jour. of Anat. and Phys., 1897.
Warthin.—Jour. of the Bost. Soc. of Med. Sciences, April, 1901.
——— Jour. of Med. Research, July, 1901.
Weidenreich.—Arch. f. Mikr. Anatomie, July, 1901.

ON THE MORPHOLOGY OF THE PINEAL REGION, BASED UPON ITS DEVELOPMENT IN ACANTHIAS.

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WITH 14 TEXT FIGURES.

The term "pineal region" is used here in a descriptively topographical sense, the observations reported concern the epiphysis, paraphysis, velum transversum, and the superior and posterior commissures chiefly in dog-fish embryos of from 11.5 mm. to 86.0 mm. The sections studied form part of the Harvard Embryological Collection, the general plan of which I have briefly described in an earlier article.[1] To facilitate confirmation of the observations the number of the embryo and of the section, as catalogued, is given for each of the illustrations.

Our previous knowledge of the pineal region in Elasmobranchs is based on the observations of Balfour (*Works*, Vol. I, p. 399 ff.) and Ehlers, **78.1,** on the development, and of Ehlers and Cattie, **81.1,** on the adult anatomy. Cattie reviews the earlier literature quite thoroughly. Gaupp's resumé, **98.3,** in the "*Ergebnisse*" is invaluable for the comparative study of the parts. The account of the development of the epiphysis proper, given by Balfour and Ehlers is essentially correct. They both saw the velum transversum, but did not specially study it. It has been more accurately figured by W. His, **92.1,** p. 361, Fig. 14. Balfour figures only the superior commissure, which he identified as the posterior, and appears not to have observed the true posterior commissure at all. Ehlers, **78.1,** has indicated both commissures in his Fig. 8, very clearly, but gives no description of their development in his text. Neither Ehlers nor Balfour mention the paraphysis, which was not recognized as a morphological constituent of the brain until much later (Selenka, **90.1**), but Balfour has recorded several of the changes in the paraphysal region. Balfour's descriptions of the pineal region are incomplete, and owing to the defective methods of the time, often inexact as to details.

In embryos of Squalus acanthias of 11.5 mm. in length[2] the changes

[1] Anatomischer Anzeiger, Bd. XVIII, pp. 128-129.

[2] The measurements of lengths are from the preserved specimens in alcohol.

in the roof of the diencephalon are just beginning. Fig. 1 represents a sagittal section, the plane of which is not true since it passes through the epiphysal anlage about in the median plane, but in the region of the hind brain passes considerably to one side so that it strikes the jugular vein, *Ve*, the first aortic arch, *Ao*, and the three "head cavities," the praemandibular, 1, the mandibular, 2, and the hyoid, 3. Miss Platt's "anterior cavity," **91.2**, 201, is also shown, and is connected in sections 88 and 89 of this series with the praemandibular cavity. In our transverse series, No. 206, section 120, the two cavities are just separated. It seems, therefore, probable that the "anterior cavity" arises

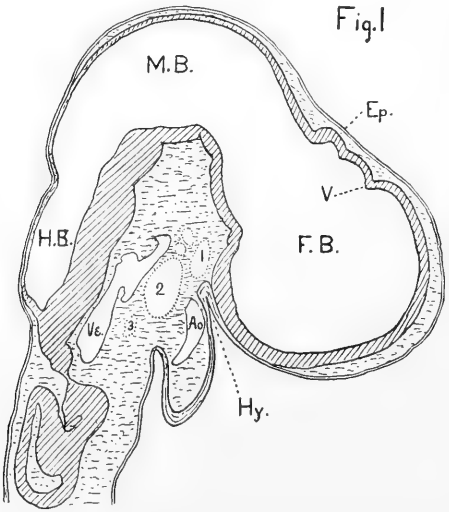


Fig. 1. Embryo of 11.5 mm. Sagittal series, No. 208, section 93. ×30 diams. (For explanation of the lettering see at end of the article.)

from the praemandibular as van Wighe found to be the case in Galeus. It may be noted that the tip of the notochord joins the wall of the praemandibular cavities, which at this stage are still united across the median line.[3] The fore-brain is already subdivided into the wider anterior prosencephalon and the narrower posterior diencephalon. The roof of the latter has two well-marked arches, of which the posterior, *Ep.*, is the anlage of the epiphysis. In connection with these arches appear on the inside of the brain wall in the section three points; of

[3] The condition of the head-cavities is described as it offers a convenient means of fixing the stage at which the differentiation of the pineal region begins.

these, the first, *V*, is the anlage of the velum; the second, which marks the boundary between the two arches, is the site for the future commissura superior; the third, which forms the posterior boundary of the epiphysal anlage is the site for the future commissura posterior—compare Fig. 5. The curve in front of the velar anlage I propose to name the paraphysal arch, on account of its subsequent differentiation; the curve between the velum and the epiphysal anlage may be termed the post-velar arch. We thus distinguish six fundamental morphological divisions in the median line of the diencephalic roof:

1. Paraphysal arch.
2. Velum transversum.
3. Post-velar arch.
4. Superior commissure.
5. Epiphysis.
6. Posterior commissure.

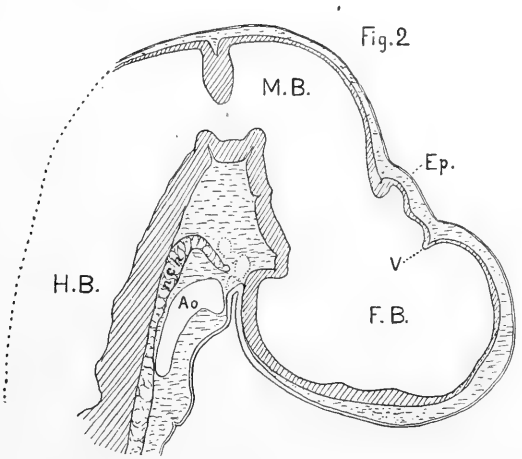


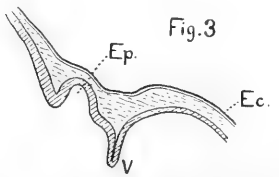
Fig. 2. Embryo of 11.5 mm. Sagittal series, No. 208, section 93. × 30 diams.

The homologues of all these parts exist probably in all vertebrates.

In the next stage, which I have, an embryo of 13.0 mm., Fig. 2, the brain is larger, and the outline of the fore-brain has been considerably modified, chiefly owing to the growth of the infundibular region. The "anterior" cavities in this embryo (No. 224, sections 99-100) seem to have no longer any connection with the praemandibular cavities, but lie further laterad. The relations are well demonstrated by transverse sections—thus No. 223, section 99, shows both the "anterior" cavity and the median connection of the praemandibular cavities. In both

series the anterior end of the notochord is bent ventralwards at a sharp angle, Fig. 2, *nch;* it tapers off gradually, and has a rounded termination, which lies close against the wall of the praemandibular cavity. The roof of the diencephalon has advanced slightly as compared with figure 1. The velum, *V*, projects further into the cerebral cavity and appears more clearly as a fold of the brain wall; in another embryo of the same length, No. 225, section 63, the projection is more marked. The epiphysal anlage has begun to deepen, *Ep.*, and immediately behind it the brain wall shows a thickening where the posterior commissure is to appear.

As shown in the figure the narrower diencephalon is marked externally by a depression between the mesencephalon, *M. B.* and prosencephalon, *F. B.* This depression can still be easily traced in embryos of 40-45 mm. but gradually disappears.



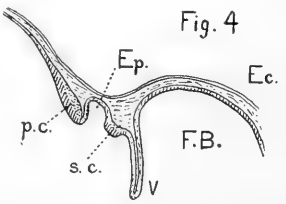
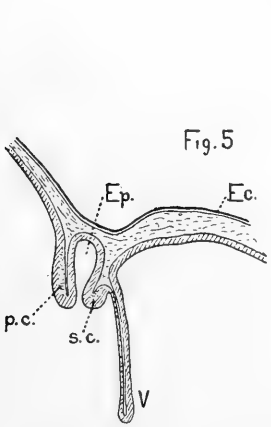


Fig. 3. Embryo of 15 mm. Sagittal series, No. 228, section 51. ×30 diams.
Fig. 4. Embryo of 18 mm. Sagittal series, No. 204, section 100. ×30 diams.

In embryos of 15 mm., Fig. 3, the epiphysal evagination, *Ep.*, has grown, as has also the velum, *V*, which now has distinctly an anterior and posterior surface; the ependymal coverings of the two surfaces lie close together so that there remains only a minimal space between the two ependymal layers. In this specimen the brain-wall is thicker in the pineal region than in both the younger and the older stages, which I have examined. Similarly the walls of the epiphysal anlage, Fig. 5, *Ep.*, are thicker than in both the older and younger stages of Figs. 4 and 6. I think these variations are not important morphologically.

The next, Fig. 4, is from an embryo of 18 mm. and shows a decided advance of the differentiation. The velum, *V*, is now a well-marked fold of the ependyma, with a narrow middle layer of mesenchyma; the fold extends across the fore-brain from side to side, and according to the current interpretation is regarded as marking the boundary between the diencephalon and the prosencephalon. The epiphysal anlage is more distinctly an evagination than before. Immediately in front of it has appeared the small but well-marked superior commissure, *s. c.*,

and immediately behind it has appeared the much larger posterior commissure, *p. c.*, the position of which—compare also Figs. 5-10—indicates that it belongs rather to the mid-brain than to the fore-brain. Opinion on this point must be reserved however until determined by an investigation that will fix the boundary between the two primary cerebral vesicles. Our present information seems to me insufficient to decide with finality this point. Both commissures are developed in the "*ectoglia*." This term I propose as the equivalent of the "*Randschleier*" of His, to designate the outermost of the three primary layers of the medullary wall.[4] In stained sections the commissures are conspicuous owing to the absence of nuclei, in contrast with the adjacent



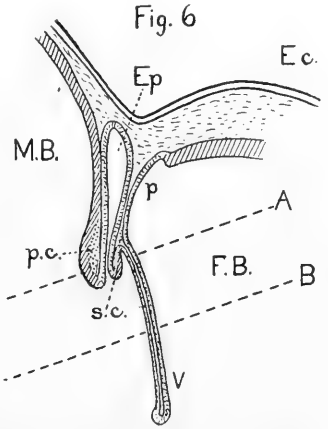


Fig 5. Embryo of 22 mm. Sagittal series, 231, section 79. × 30 diams.

Fig. 6. Embryo of 28.0 mm. Sagittal series, 233, section 122. × 30 diams. *A*, approximate plane of the section represented in Fig. 11; *B*, approximate plane of the section represented in Fig 12.

tissue of the brain-wall, which is densely crowded with nuclei. The cross sections of the transverse nerve fibers are sharply marked in the sections.

Embryos of 22 mm.—4 mm. longer than the last—show a decided growth of all the parts, and the growth is downward, *i. e.* towards the interior of the brain. The ectoderm, *Ec.*, is still a thin layer, and the mesoderm between it and the brain-wall has increased a little in thick-

[4] Upon the fundamental morphological importance of these three layers I have insisted in my *Human Embryology*, p. 616. The entire structure of the adult brain and cord should be stated in terms of these three layers.

ness; accordingly the outer surface of the mid-brain and of the fore-brain, *F. B.*, also the summit of the epiphysis, *Ep.*, are all nearly as close to the outer surface of the head as before, while on the contrary the orifice of the epiphysis, the posterior commissure and the superior and the edge of the velum are all much more remote from the surface than before. Comparison of the successive stages shown in Figs. 4-10 will suffice to demonstrate that increasing downward extension is the method of development by which all these parts elongate. This comparison will also demonstrate that the summit of the epiphysis remains quite close to the ectoderm, while the continued development of the mesoderm is increasing the distance between the epidermis and the mid-brain and fore-brain both, so that the epiphysis comes to project more and more above the level of the brain. Two further characteristic features are illustrated by Fig. 5, *first* the small growth of the post-velar arch, so that the distance between the base of the velum and the orifice of the epiphysis is barely more than sufficient for the superior commissure. It is probable that the small size of the post-velar arch is a special characteristic of the elasmobranch type. In other vertebrates the post-velar arch has considerable extension in the sagittal direction, as in Accipenser according to Kupffer, and in Necturus, Fig. 13, and Gallus, Fig. 14. *Second*, the ependyma on the posterior side of the velum has grown thinner, except that towards the inferior edge of the velum it thickens and is then reflected over the edge onto the anterior surface. Finally it must be noted that up to this stage the velum has elongated more rapidly than the epiphysis, after this stage on the contrary the epiphysis elongates much more than the velum.

In embryos of 28.0 mm. the permanent relations are already clearly indicated. The most important advance has been the thickening of the wall of the fore-brain, except as seen in the sagittal sections towards the velum, where the wall corresponding to the area of the paraphysal arch remains thin. The differentiation of this arch therefore occurs quite late, and may perhaps be best described as resulting from an arrest of the histological development, which just in front of the arch progresses rapidly, there causing the brain wall to thicken, and to change into nervous tissue proper. Owing to the continued downgrowth of the parts a deep fold or cleft is formed between the mid-brain, *M. B.*, and fore-brain, *F. B.* In and near the median plane the space of the cleft is almost filled by the epiphysis, as shown in the figure—the wall of the epiphysis being almost in contact with the wall of the mesencephalon behind, and with the paraphysal arch in front. Laterally the cleft between the two vesicles is filled only with mesen-

chyma, as can be seen in Fig. 11. Comparison with Fig. 5 shows that the velum has elongated, but comparatively much less than the epiphysis. Both commissures have grown, causing each a local thickening of the brain-wall. Our notions of the structure may be completed by the examination of cross sections, compare below Figs. 11 and 12.

In embryos of 34.0 mm. we find that there has been an obvious growth of all the parts, and in addition we note:—1, that the paraphysal arch is more accentuated, and has changed in form so that it has now be-

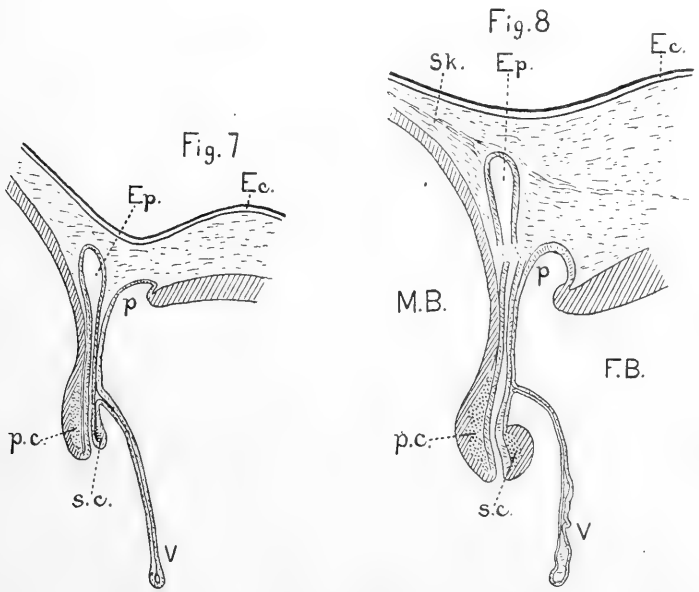


Fig. 7. Embryo of 34.0 mm. Sagittal series, 362, section 172. × 30 diams.
Fig. 8. Embryo of 40.5 mm. Sagittal series, 370, section 169. × 30 diams.

gun to bend upwards, so as to reach above the level of the fore-brain in front of it; 2, the ependyma on the anterior side of the velum has thinned out, so that it is about the same as the ependyma upon the posterior side; 3, the epiphysis, *Ep.*, is clearly differentiated into a terminal enlargement and a narrower stalk.

In an embryo of 40 mm., Fig. 8, we may point out the general growth, which has continued in all the parts, and besides must direct attention to the following special points:—the marked thickening of the nervous portion of the fore-brain wall, the upward protuberance of the paraphysal arch; the decided curvature of the velum, and its

peculiar insertion, which causes it to appear almost (in these sections) like an appendage of the epiphysal stalk; the growth of the post-velar arch between the base of the velum and the superior commissure, *s. c.;* and finally the well-marked thickening of the mesencephalic wall.

I have examined also sagittal sections of the pineal region of embryos of 50 and 60 mm., but, deeming it superfluous to present pictures

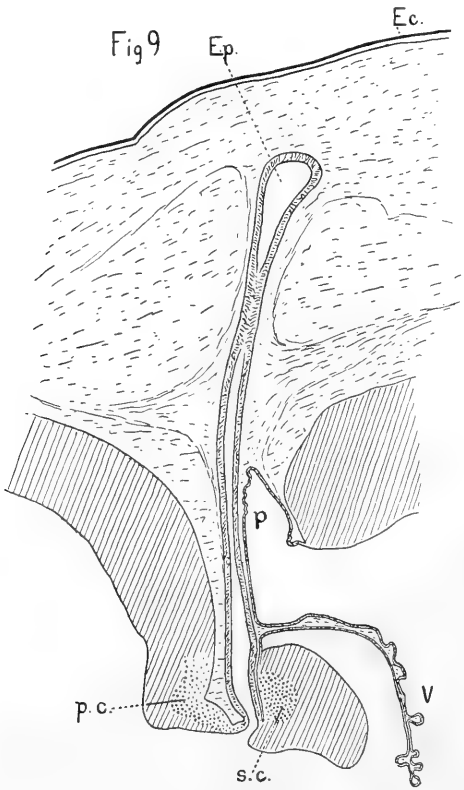


Fig. 9. Embryo of 70 mm. Sagittal series, 421, section 293. ×30 diams.

of them, will pass at once to Fig. 9, taken from an embryo of 70 mm. The nervous tissue proper of the mid-brain and fore-brain has grown very much so that these parts now form a striking contrast owing to their increased thickness with the ependymal covering of the pineal region, that is to say of the paraphysal arch, *P*, and velum, *V*, and the epithelium of the epiphysis, which, however, is considerably thicker than the velar and paraphysal epithelium. Around the superior com-

missure, *s. c.*, there is also a considerable accumulation of differentiated nervous tissue, indicated in the figure by shading with lines. This material does not extend across from side to side but is interrupted by a deep and rather narrow cleft in the morphological median plane—see section 299—as can be readily observed in transverse and frontal sections of this and earlier stages. The section figured passes through the mouth of the epiphysis but does not pass through the cleft mentioned, hence the illustration gives a somewhat false impression. The thickenings in question are continuous with the wall of the fore-brain; they were termed *tubercula intermedia* by Gottsche, **35.1,** walls of the thalamencephalon by F. M. Balfour, and by recent writers are often referred to as the *ganglia habenulæ*. I have not pursued the study of these structures further. I will only remark in passing that the term *ganglion habenulæ* does not appear to correspond with any morphological conception sufficiently clear to be valuable in comparative anatomy. During the earlier stages the mesodern between the epidermis, *Ec.*, and the brain has been steadily growing and has now reached considerable proportions, but the summit of the epiphysis, *Ep.*, still lies relatively near the outer skin and the organ consequently projects far above the brain; it has begun to curve forward preparatory to its elongation rostrad. As regards the superior commissure, *s. c.*, it is noteworthy that it is now nearly as large as the posterior. As we ascend the vertebrate series the posterior commissure increases in size and importance, but the superior commissure is persistent occurring even in mammals. The velum, *V*, has distinctly the character of a choroid plexus, being rich in blood vessels, and bearing irregular villous outgrowths, the beginnings of which can be seen in Fig. 8. The velar villi are much more developed laterally and their formation is spreading forward around the sides of the paraphysal arch, a fact which I regard as of fundamental importance for our final morphological interpretation.

The last stage I have been able to investigate is an embryo Acanthias of 86.0 mm. which is in a somewhat imperfect state of preservation, the epidermis being in part lost, entirely so in the part drawn, although indicated in the figure; the brain had shrunk a little; and as the ependyma had withdrawn from the mesenchyma on both sides of the velum that structure appears abnormally thick, but there seem to be no important distortions. The anlage of the skeleton, *sk*, is now clearly defined in the mesenchyma on the same level as the upper vesicular end of the epiphysis, *Ep.;* the stalk of the same is bent and has begun to elongate forwards above the brain thus making a distinct approach towards the adult condition described by Ehlers and Cattie. The para-

physal arch forms a distinct evagination, *P*, with thin walls and its top is irregular as if the walls had formed the anlages of new outgrowths. I am thus led to believe that the arch has not at this stage yet completed its differentiation, but on the contrary is about to form the true paraphysis, or paraphysal gland as a local development of the arch proper. My efforts to obtain more advanced stages having failed I must leave the decision to future observations. My belief, or better said—supposition, is confirmed by the fact that in amphibians and birds the

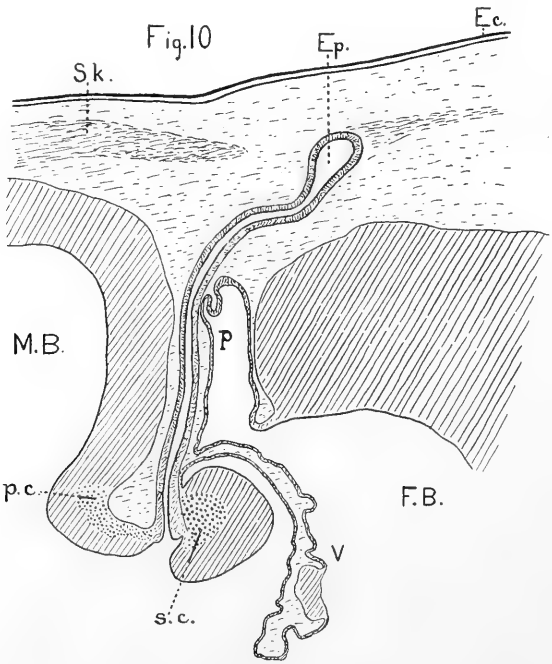


Fig. 10. Embryo of 86 mm. Sagittal series, 426, section 293. ×30 diams.

paraphysal gland is an appendix of the arch—see below. The velum, *V*, has now distinctively the character of a choroid plexus being very irregular in the form of its surface, rich in blood vessels, covered by a thin ependyma and projecting far into the cavity of the brain. Laterally the projections from its surface are much more developed, and as the organ has grown forward alongside the median paraphysal arch, it has produced what we can now easily identify as the plexus of the lateral ventricles. These plexuses are therefore to be interpreted morphologically as secondary modifications or appendages of the primary

velum transversum—compare below, Fig. 12. The superior commissure, *s. c.*, is now in the area of its cross section fully equal to the posterior commissure, *p. c.*, and must be regarded as of great morphological importance. The nervous portion of the wall of the fore-brain shown in the figure in front of the paraphysal arch is very thick.

The ependymal covering of the velum early develops a superficial coat, which appears lightly colored in stained sections. In embryos of 22 mm. (stage of Fig. 5) this border is easily seen on the anterior surface of the velum, but not on the posterior surface. In embryos of 28 mm. the border is conspicuous, but is thinnest at the base of the velum, and gradually thickens toward the inferior edge. The nature of the border is uncertain; the appearance is probably not due to cilia, but suggests rather the formation of secretory spherules, such as I have recently discovered in the cervical glands of the human uterus, the Wolffian body of the pig, and the kidney of the frog. These spherules are formed from the free ends of the cells, and resemble those which, as Mingazzini has shown, are formed on the inner ends of the absorbing epithelial cells of the mammalian intestine. I wish, however, not so much to offer a definite interpretation of the border, as to suggest a line of investigation.

In order to render clearer the relations of the velum Figs. 11 and 12 have been added. These are both from a series of transverse sections of an embryo of 28 mm., or of the same length as the embryo from which Fig. 6 is taken. The plane of Fig. 11 is approximately that of the dotted line A in Fig. 6. It passes, therefore, through the mid-brain, *M. B.*, and fore-brain, *F. B.* Next the former is the oval section of the stalk of the epiphysis, *Ep.*, crowded into the space between the two commissures. The posterior commissure, *p. c.*, apparently belongs wholly to the mid-brain, as can be seen in the sagittal sections also. The development in Necturus, in the chick, and in the rabbit and pig also indicates that the posterior commissure belongs to the mesencephalon. When it is further remembered that its fibres in man are of mesencephalic origin, we must conclude that the traditional description of the commissure as situated in the roof of the third ventricle is erroneous, since the commissure does not belong to the diencephalon. Returning to Fig. 11, the superior commissure, *s. c.*, runs in the cerebral tissue of the post-velar arch—compare Fig. 6—between it and the velum proper, *V*, appears the narrow space of that arch; laterally this space curves towards the mid-brain. The velum, *V*, stretches from side to side of the brain, it consists of a thin central layer of mesenchyma continuous laterally with the mesenchyma surrounding the

brain, and of two layers of ependyma, of which the posterior is the thinner.

The plane of Fig. 12 is approximately that of the dotted line B in Fig. 6. The velum, *V*, belongs to the fore-brain, dividing the cavity thereof into a large anterior (in Fig. 12 lower) chamber and the much smaller chamber of the post-velar arch, which is extended far out laterally, the lateral cavities being slit-like; the lateral post-velar slits are bounded in front (below) by a thin ependyma, and posteriorly (above) by the thick brain wall of the *tubercula intermedia* in which one readily

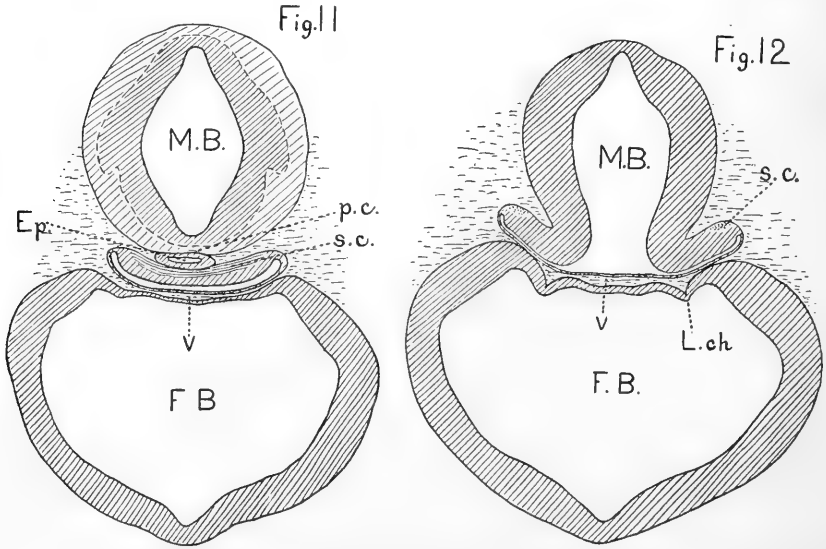


Fig. 11. Embryo of 28.0 mm. Transverse series, 232, section 130. The plane of the section is indicated by the dotted line A in Fig. 6. × 30 diams.

Fig. 12. Embryo of 28.0 mm. Transverse series, 232, section 163. The plane of the section is indicated by the dotted line B in Fig. 6. × 30 diams.

distinguishes the lateral prolongations, *s. c.*, of the superior commissure. As the tubercula belong to the fore-brain their junction with the mid-brain, which is marked externally by the apex of a deep furrow, indicates the division line between the first and second primary cerebral vesicles. From such a section as Fig. 12 one might be easily led to interpret the velum not as the division between the diencephalon and prosencephalon, but between mid- and fore-brain, and further to interpret the *tubercula* as appendages of the mid-brain. Attention should be paid to the two lateral projections, *L. ch.*, of the ependyma on the

anterior surface of the velum, because these projections not only fix the lateral boundaries of the paraphysal arch but also are the anlages of the choroid plexus of the lateral ventricles. These anlages from this stage on rapidly increase both in size and in complication of form.

I wish now to add certain comparisons of the structures described with those of other vertebrates, particularly with a view of determining the homologies of the paraphysis, velum, and superior commissure. Since Selenka's original brief announcement, **90.1,** of the recognition of the paraphysis as a distinct vertebrate organ, little has been added to our morphological conception of it. It is completely ignored by the standard German and English text-books, and Prenant in his Traité

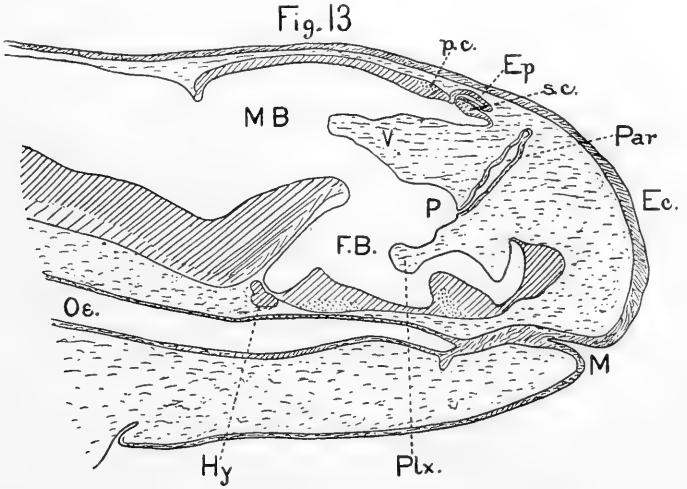


Fig. 13. Necturus maculatus of 18 mm. Sagittal series, No. 23, section 90. × 30 diams.

d'Embryologie is the only author, known to me, who has attempted a systematic analysis of the scattered observations.

The paraphysis is a gland developed by a local evagination of the epithelium of the paraphysal arch, and so far as known never is differentiated as a sensory organ.

As I have found no paraphysis in Acanthias embryos up to 86 mm., we must have recourse to other types. I will mention first Necturus, Fig. 13, and the chick, Fig. 14, as on these two forms I have made original observations. In a Necturus of 12.0 mm. (Sagittal series 49, section 60) the stage of the pineal region corresponds more or less to that of acanthias shown in Fig. 3; the epiphysis is evaginated, and there is

a well defined paraphysal arch in front of the velum and post-velar arch behind it. In a Necturus of 15.0 mm. (Sagittal series 79, section 84) the paraphysis is a narrow elongated evagination from the arch, and so appears again in embryos of 18 mm., Fig. 13. In embryos of 21 mm. the wall of the paraphysis is irregular, and in a larva of 26.0 mm. the anlages of the paraphysal gland tubes are easily recognized (Sagittal series 377, section 126).

In the chick the paraphysis is developed very late and at seven days is a small nearly hemispherical evagination, Fig. 14, *Par.*, formed from the paraphysal arch, *P*, quite near the rudimentary velum, *V*. A day later the epithelium is thicker and irregular as if the glandular tubules were beginning to form.

In Petromyzon Burckhardt figures a *small* evagination, which is probably the paraphysis, but his figure does not show the limits of the paraphysal arch. In various forms it is known that the paraphysis arises as a small evagination, which appears just in front of the velum and rather late in development as a local outgrowth. See for example Kupffer's observations on Accipenser; the observations of de Graaf, Burckhardt, Eycleshymer and others on Amphibia, those of Dendy and Burckhardt on reptiles. In all these cases there is a comparatively wide paraphysal arch, and a small paraphysis. The two things have heretofore not been distinguished so that there is considerable confusion in the descriptions.

The existing observations render it probable that the paraphysis is a true gland, the main evagination serving as the duct, while the secondary tubules are the secretory portions. Certainly the type of organization is that of a gland and not of a sense organ, and whenever the adult paraphysis has been found and studied it has presented the same plan of structure. Its secretion must of course pass into the cavity of the brain, so that functionally it is comparable to the glandular epiphysis in birds, and the infundibular gland of all vertebrates. One may suppose that these three glands supply some substances, which are useful to the nervous system, and that they are somewhat comparable from the physiological standpoint to the ductless glands at least in respect to the fact that their secretion has no direct open channel of escape from the body.

The velum probably is characteristic of all vertebrates. In elasmobranchs the post-velar arch remains small, hence the velum seems to arise later very close to the mouth of the epiphysis. In ganoids the post-velar arch is well developed, hence the velum is inserted quite far in front of the epiphysis. As regards the teleosts the data for a satis-

factory interpretation seem to me lacking. In amphibia (as in necturus) there is at first a well-defined velum; the mesenchyma within the velum increases greatly in amount and converts the velum into a broad structure, Fig. 13, *V*. I have been unable as yet to follow the growth of the velum in sufficient detail, but it is probable that, as the velum expands, the post-velar arch is incorporated in it and disappears as a separate region. The enlarged velum projects backward, extending

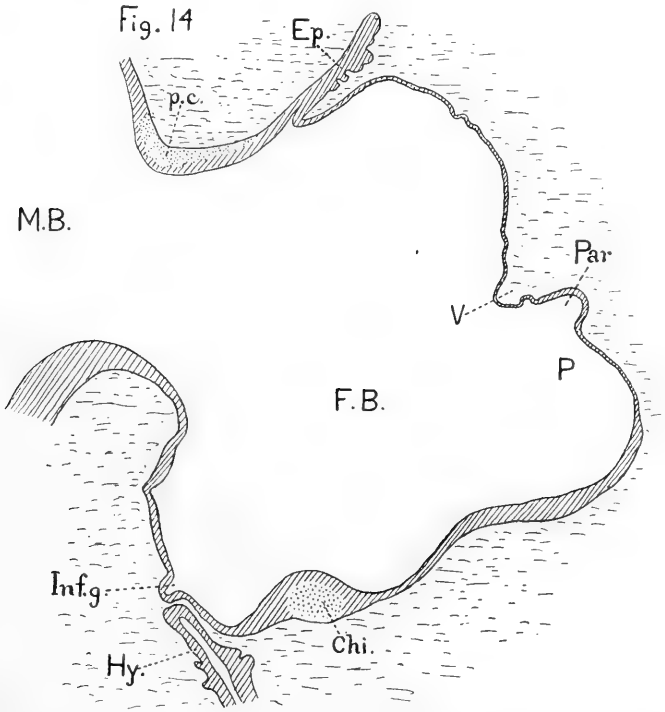


Fig. 14. Chick embryo of about 7.0 days. Sagittal series, 354, section 204. ×30 diams.

even into the mid-brain, and meanwhile expands laterally around and in front of the paraphysal arch, until its wings meet across the median line, Fig. 13, *pl. x*, to form the anlage of the anterior plexus. The entire expanded velum gives rise to the choroid plexus (supra-plexus of American writers), which accordingly surrounds the paraphysis. In the adult the paraphysis has become a gland of complex structure, as has been so well described by Francotte, but by most writers has been confused with choroid plexus. In *Rana halecina* I find the adult

paraphysal gland very clearly distinguishable by, 1, the character of its epithelium, 2, its tubular structure and 3, its apparently sinusoidal circulation, from the choroid plexus proper, in the midst of which lies the orifice of the gland. In reptiles the velum has been identified and its morphological importance for the class emphasized by Burckhardt (*Anat. Anzeiger*, IX, 320). In birds, Fig. 14, the velum, *V*, is almost rudimentary in its median portion, though very broad, and it merges without recognizable boundary into the very broad post-velar arch, which can already be identified as the anlage of the *tela choroidea*. As regards mammals further investigation is necessary, both to determine the history of the velum and of the paraphysis, if that organ is present in the placental, as Selenka states it to be in marsupial, mammals. In both birds and mammals the lateral portions of velum, *i. e.* the choroid plexus of the lateral ventricles is highly developed. It thus appears that as we ascend the vertebrate series there is first a broadening of the velum, and an increase of its lateral development, then occurs a further reduction and flattening out of the velum, and a much greater growth of the lateral plexus.

The superior commissure is a remarkably constant structure in the vertebrate brain, and must, since it persists in all vertebrate types, be regarded as a fiber tract of fundamental morphological importance. H. F. Osborn, **84.1**, 268, was the first to demonstrate the homologies, wide occurrence and topographical relations of the tract, and to apply the name "*supra commissura*." So far as I am aware, it has not yet been recorded in birds, but a more thorough search will perhaps lead to its discovery in that class. It has been found in representative types of all other vertebrate classes, including mammals, for I have observed it in embryos of rabbits, pigs and cats. In all cases it develops later than the posterior commissure—in mammals much later, and is at first much smaller than the posterior commissure. It acquires a large size in acanthias and perhaps other fishes, it attains less size in amphibians and is proportionately smallest in mammals. Its position is very constant as it is always situated immediately in front of the orifice of the epiphysis and at the outer surface of the brain wall.

The posterior commissure belongs morphologically to the mid-brain, not to the fore-brain. For the present the epiphysis may be accepted as marking the posterior limit of the fore-brain.

The pineal region develops a series of structures, which, from their anatomical characteristics, appear to be directly concerned in the formation of the fluid in the cavities of the brain. We may assume that the choroid plexus supplies the main bulk of the fluid, but the gland-like

organization of the epiphysis and of the paraphysis indicates that they supply by secretion special chemical substances to the encephalic fluid. It remains, therefore, for the physiologist to investigate these glands and to determine how far their action is indispensable to the brain, and for the pathologist to investigate the possible relations of diseases of these glands to cerebral disorders. The pathological study of these organs seems to me all the more important, because I regard as reasonable the anticipation that the paraphysal gland will be discovered in man.

EXPLANATION OF THE LETTERING.

Ao., aorta.
Chi., optic chiasma.
Ep., Epiphysis.
Ec., Ectoderm.
F. B., fore-brain.
H. B., hind-brain.
Hy., hypophysis.
Inf. g., infundibular gland.
L. ch., lateral choroid.
M. B., mid-brain.
M., mouth.
nch., notochord.
Oe., oesophagus.
P., paraphysal arch.
Par., paraphysis.
p. c., posterior commissure.
Plx., choroid plexus.
sk., anlage of skull.
s. c., superior commissure.
V., velum.
Ve., jugular vein.
1, praemandibular head-cavity.
2, mandibular cavity.
3, hyoid cavity.

BIBLIOGRAPHY.

CATTIE, J. T., **81.1.**—Verglijkend-anatomische en histologische Onderzokingen van der Epiphysis cerebri der Plagiostomi, Ganoidei en Teleostei. Transl. Arch. Biol. III, 101-194, Pls. IV-VI.

EHLERS, **78.1.**—Die Epiphyse am Gehirn der Plagiostomen. Zeitschr. wiss. Zool., XXX, Suppl., 607-634, Taf. XXV-XXVI.

GAUPP, ERNST, **98.3.**—Zirbel, Parietalauge und Paraphysis. Ergebn. Anat. Entwick-ges. VII, 208-285.

GOTTSCHE, C. M., **35.1.**—Vergleichende Anatomie des Gehirns der Grätenfische. Müller's Arch., p. 453.

HIS, WILHELM, **92.1.**—Zur allgemeinen Morphologie des Gehirns. His' Arch., 1892, 346-383.

HIS, WILHELM, **92.2**—Die Entwickelung der menschlichen und thierischen Physognomien. Arch. Anat. Physiol., Anat. Abth., 1892, 384-424.

OSBORN, HENRY F., **84.1.**—Preliminary observations upon the brain of Menopoma. Proc. Acad. Nat. Sci., Philadelphia, 1884, 262-274, Pl. VI.

PLATT, JULIA B., **91.2.**—A contribution to the morphology of the vertebrate head, based on a study of Acanthias vulgaris. Journ. Morphol., V. 79-112, Pl. VI.

SELENKA, EMIL, **90.1.**—Das Stirnorgan des Wirbelthiere. Biol. bbl. X, 323-326.

THE SPERMATOGENESIS OF DESMOGNATHUS FUSCA.

BY

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WITH 4 PLATES.

The work of which this paper is the outcome, was undertaken in the winter of 1896. *Necturus maculatus* was the form first chosen, but it was soon abandoned for the salamander *Desmognathus fusca*, which from its abundance, availability at all seasons of the year, and the size and structure of its testis, seemed a more suitable object for the work in hand. A preliminary note on the divisions of the spermatocyte was published in 1899. In November, 1900, the drawings, photographs and the larger portion of the preparations covering what would have been the first part of this article were destroyed by the burning of the histological laboratory of Cornell University. The plates (published herewith) and the portion of manuscript discussing the divisions of the spermatocyte were saved, and it has seemed best to publish that portion without waiting to repeat the work of the first part, which would take several years.[1]

When this investigation was begun, the need for careful work on the spermatogenesis of Amphibia was quite apparent. Research on the spermatogenesis of Amphibia had been largely confined to the European salamander, *Salamandra maculosa*, and to that form alone had a monographic treatment been accorded, in the papers of Flemming, 87, vom Rath, 93, and Meves, though his final paper did not appear until 1897. Since this work was undertaken, however, McGregor, 99, has published a monograph on the spermatogenesis of *Amphiuma*, and Eisen, 00, has brought out an extensive article on *Batrachoseps*, another American salamander.

The results of Flemming and vom Rath have been reviewed by Meves, 97, and need not be discussed here in any detail. Flemming's paper,

[1] The writer wishes to acknowledge gratefully the help which has been given him with the manuscript and preparations by S. H. Gage, head professor of the department, and by M. Hempstead and C. F. Flocken, students in the laboratory.

which appeared in 1887, is classical, not only as a contribution to the knowledge of spermatogenesis, but of mitoses in general. In Salamandra Flemming recognized the period of multiplication of the spermatogonia, followed by the period of growth to form the spermatocyte, which he believed divided twice, forming medium-sized and then small cells. The latter he apparently thought divided again,[2] there being thus three generations of cells resulting from the division of the spermatocytes. In these divisions were recognized and named two divergent types of mitosis, "homotypic" and "heterotypic"—terms and types widely known in spermatogenesis work. The divisions of the spermatogonia were found to be homotypic, those of the spermatocyte of the first order characteristically heterotypic, though homotypic divisions also occurred; the division of the spermatocyte of the second order was believed to be heterotypic and homotypic equally.

In the interval between the appearance of Flemming's paper, 87, and vom Rath's, 93, Weismann, 91, had published his prophecy that in the process of spermatogenesis and oögenesis a "reducing" division would be found to take place.

The results of Haecker, Henking, Rückert and vom Rath appearing soon after, brought an apparent confirmation of the correctness of this prediction, in the forms investigated by them. Vom Rath approached the investigation of Amphibian spermatogenesis (Rana, Salamandra) fresh from his results in Gryllotalpa, in which he had described tetrad formation. To the three generations described by Flemming he added a fourth in which tetrads were formed and which were distributed in two more divisions, as he believed to be typically the case, making six generations in all. Vom Rath's investigation was evidently undertaken to see if tetrad formation did not also obtain in Amphibia, and he was led to suspect the correctness of this view by certain descriptions and figures of Flemming in which tetrad formation was apparently shown, though regarded as abnormal.

Meves' published work on the spermatogenesis of Salamandra began in 1891, his main paper appeared in 1897, followed the next year by a paper on the transformation of the spermatid into the ripe spermatozoon.

[2] The statements of Flemming on this point do not seem to leave his interpretation entirely clear. In his schematic table of the cell generations, only two generations are given as descendants of the spermatocyte, and the following words convey the same impression "Mehr Tochtergenerationen der grossen Zellen als zwei scheinen mir den vorfindlichen Zellengrossen, nicht vorzukommen" (p. 401). Nevertheless, to Meves, Flemming personally stated that he had believed there were three generations of cells.

His results did not sustain those of vom Rath; no tetrad formation was found to occur, and there were but three cell-generations instead of six. On the other hand, the divisions of the spermatocytes he found to be equation divisions, the first heterotypic in character, the second homotypic, not mixed as Flemming had thought; furthermore, the spermatocytes of the third generation did not divide as Flemming seems to have supposed.[3] His paper, 97, dealt with the entire spermatogenesis, the structure and divisions of the spermatogonia, as well as the growth and divisions (two) of the spermatocyte, involving a consideration of reduction. The papers of McGregor and Eisen, which will be considered in the body of this article, supported the results of Meves (as far as the purposes of this paper are concerned). These five papers include the more exhaustive investigations of Amphibian spermatogenesis. Minor papers contributing to our knowledge but not dealing with the question of reduction need not be considered here, as also the work of older writers of historical interest in the analysis of the structure of the Amphibian testis. Of the publications on Amphibian oögenesis, the only one that is of importance for the purposes of this article is the paper of Carnoy and Lebrun.

DESMOGNATHUS FUSCA.

The form chosen for this investigation is one of the most abundant salamanders in this locality (Ithaca, New York, U. S. A.), and in eastern United States generally; it is likewise the most abundant species of the genus. In 1866 the *Desmognathidæ* were recognized by Cope as a distinct family, represented by a single genus, to which have since been added the genera *Thorius*, *Typhlotriton*, and finally, *Leurognathus*.

Desmognathus, in habits is semi-aquatic, living at the edge of swiftly running brooks, especially near or at springs. It chooses concealment under stones at the edge of the stream, or among the pebbles of coarse gravel, saturated with water; dry ground, and also deep water in the bed of the stream, it apparently avoids. If confined in an aquarium of still water, it will crawl up the sides so that its head and a portion of its body are out of water; if prevented from thus gaining access to the air, it speedily dies.

It is said to be nocturnal in its feeding habits, Wilder, 99, wandering forth in search of its food, which consists of small insects (beetles, etc.) and their larvæ.

Of its breeding habits and development very little is known. The

[3] See note [2], p. 100.

eggs are laid some time in the summer. Wilder has found eggs laid in captivity, June 1st; Sherwood, 95, on the other hand, has found eggs from July to October, so that there is probably considerable individual variation in the time of ovulation. *Desmognathus* eggs have been found at Ithaca during July and August. Recorded observations are insufficient for a more exact determination of the time of ovulation. The development of the form is known only in its grossest outline, but the very interesting statement has been made that cleavage in *Desmognathus* is meroblastic, Wilder, 99, and this statement has also been guardedly made of a member of an allied family, *Autodax lugubris*, Ritter, 99. The eggs are laid under stones near the stream in a hollow in the mud and are connected together by albuminous cords, uniting them in a group. The female remains with the eggs, which lie in a mass at her side, according to my observations, not wrapped about her body as Wilder has stated to be the case.

Mating habits.—Nothing definite is known of the mating habits of *Desmognathus*. The investigations, especially of Zeller and Jordan, have made us well acquainted with these phenomena in the genus *Salamandra* and in the genera of the allied family of the *Pleurodelidæ*, where they consist in the deposition of a spermatophore by the male, over which the female passes while her cloacal lips, coming in contact with the mass of spermatozoa, either actively grasp it, or to them the spermatozoa adhere and enter later by their own activity. Within the cloacal chamber the spermatozoa become ensconced in tubules constituting spermathecæ, in which they remain until the time of ovulation. Fertilization in *Salamandra* (Triton and *Diemyctylus*) takes place in the spring, and is preceded by a mating. In Triton and *Diemyctylus*, at least, there is occasionally an autumnal mating, the entire winter intervening before ovulation.

As compared with what is known of the mating habits of these forms, our knowledge of the same phenomena in *Desmognathus* is scanty, indeed. I know of no published observation on this form which, from its retiring mode of life and (presumably) nocturnal habits, is difficult to observe. The probability is strong, however, that the same mode of mating occurs in this form, since in the male *Desmognathus* are present in a well developed condition the same three cloacal glands found in forms whose mating is known and which undoubtedly produce the secretion that constitutes the body of the spermatophore. Likewise, the spermatheca is present in the cloaca of the female, and is a much more highly specialized organ than in the *Salamandridæ* and *Pleurodelidæ*. In specimens of *Desmognathus* taken in the fall, winter, spring and summer, it was

found to be quite full of spermatozoa. This, of course, affords no clue to the time of mating or ovulation. Examination of the testis shows that the mass of spermatozoa leave it in the early fall, so that it contains but few ripe spermatozoa. But the ducts are well filled and in the spring the cloaca as well contains a mass of spermatozoa upon a jelly-like base, presumably a spermatophore. This suggests a spring mating with quite probably a fall one also.

Nomenclature and Spermatogenetic Cycle.

It is perhaps hardly necessary to define the meaning of the terms that will be used in this paper as applied to the generations of cells in spermatogenesis, so universally have La Valette St. George's, 76, names of (a) spermatogonia, (b) spermatocytes of the first order, (c) spermatocytes of the second order, (d) spermatids and (e) spermatozoa, been used in all recent work; and so well recognized are the corresponding periods which they characterize—(a) a period of multiplication, (b) a period of growth or maturation, (c) a reduction period, sometimes spoken of as the maturation period, and (d) the period of transformation, sometimes also called the maturation period. Meves has further divided the spermatogonia into large and small spermatogonia, a division which, as a matter of convenience, seems to be helpful, though McGregor's terms of primary and secondary spermatogonia are preferred by the writer.

In *Salamandra*, as Flemming early pointed out, the spermatogenesis forms an annual cycle in which in adaptation to the breeding habits of the animal, the stages succeed each other in chronological order and characterize certain periods of the year. After mating in the spring a multiplication of the residual spermatogonia takes place which continues during the months of April, May and June. During late spring and early summer occurs the period of growth and maturation of the spermatogonia to form the spermatocytes; during June, July, and also into August, the reducing divisions are taking place, followed by the transformation of the spermatids into spermatozoa in August and September. The testis in the winter is filled with ripe spermatozoa, which are emitted in the following spring, when another yearly cycle begins.

In *Desmognathus* the cycle is shifted but slightly, so that the characteristic periods occur at nearly the same times as in *Salamandra*. The testis of animals killed in the autumn (October, November) contain none or but few ripe spermatozoa; the spermatogonia are, however, dividing and the secondary spermatogonia have entered upon their

period of growth to form the spermatocytes. Mature and dividing spermatocytes occur sporadically, but seem to be those left over from the preceding summer. During the winter a sluggish division of the spermatogonia is taking place and a few lobules containing spermatozoa or spermatids are usually encountered. There does not seem to be much activity in the organ during the cold months, and there is practically no increase in size, but in the spring mitoses become numerous and more cysts of spermatogonia are found in stages of cell division. Growth to form the spermatocytes also begins anew, continuing well into July. During June and July, especially in July, divisions of the spermatocyte are taking place, and multiplication of the spermatogonia has practically ceased. The transformation of the spermatogonia into spermatocytes stops in June, while the transformation of spermatids into spermatozoa, beginning in June or July, extends through August into September. Most of the spermatozoa leave the testis in the early fall and the cycle begins again.

The spermatogenetic cycle of *Desmognathus* corresponds closely, therefore, with that of *Salamandra*, differing in the extrusion of the mass of the spermatozoa in the fall, so that the cycle may be said to begin then instead of in the spring, though there is no great activity until spring. The months when the processes of spermatogenesis are actively taking place are the spring and summer months. During April, May and June transformation of the spermatogonia into spermatocytes is predominant; the divisions of the spermatocyte characterize July, and the transformation of the spermatid, August. The processes, however, overlap widely with doubtless considerable seasonal and individual variation.

The Testis.

This organ is quite elongated and attached to the dorsal wall of the abdominal cavity by a mesorchium in which are the *vasa efferentia*, blood-vessels, etc., as in other Amphibia. It is highly pigmented, as is also the spermatic duct, the pigmentation diminishing with the expansion of the organ as spermatogenesis proceeds.

The structure of the Amphibian testis has been investigated by several writers, among them von Wittich, Leydig, Bidder, Spengel, La Valette St. George, 76, and Hoffmann, 78, all of whose papers appeared prior to 1880. Of these the papers of Spengel and Hoffmann appear most valuable. Spengel, in his analysis of the structure of the organ, recognized "capsules" (containing sperm-producing cells), connected with a collecting duct by branch-tubules. According to the relations and ar-

rangement of the capsules in regard to the collecting duct, three general types of structure were found: (1) with a longitudinal collecting duct in the center and the capsules radially arranged, (2) with the longitudinal canal superficial and the arrangement of the capsules fan-like, (3) the capsules short (more spherical) and terminating the divisions of the richly branched collecting duct. Between these extreme types, however, are transitional forms even in different parts of the same testis, as, for example, the central longitudinal canal becoming superficial. Hoffmann extended Spengel's work, adding also to the knowledge of the development of the organ. La Valette St. George, 76, in his well-known paper, carried the analysis of the structure a step farther. The testis is made up of "tubules" (capsules of Spengel), which may be hollow or not, and these "tubules" are made up of "follicles" enclosing "spermatocysts"—clusters of cells formed from a single original cell by division.

Meves recognizes the occurrence of the "cysts," which are arranged together in the form of a "thick-walled vesicle enclosing a central cavity. . . . Therefore, it is not proper to speak of tubules (referring to La Valette) here." These vesicles again are the tubules of La Valette, the capsules of Spengel.

Far more appropriate seems the designation of "lobule," which will be the term employed in this article. These divisions or lobules, separated from each other by connective tissue, are the structural units of the organ, and each is connected with the central collecting duct by a short tubule. Further, their homology with the lobules of the mammalian testis seems probable. Hoffmann has found that the collecting duct with its branches is developed from the tubules of the mesonephros, suggesting, therefore, their homology with the *rete testis* and *vasa recta*. The lobules of the Amphibian testis are not differentiated into tubules, though in some forms (*e. g.*, Bufo) the structure of the tubule of the mammalian testis is suggested.

The testis of *Desmognathus* combines the first and second of Spengel's three types, there being a longitudinal collecting duct, which is centrally located save at the ends of the organ, where it becomes nearly or quite superficial. About this collecting duct the lobules are placed radially, each connected with it by a short cord of cells whose arrangement in the form of a tubule is more or less evident. The form of the testis varies greatly with the season of year, due to the changes in the spermatogenetic cycle. In general, it is enlarged in the center and tapering at both ends, the enlargement and shape of any part being caused by the state of development and therefore size of the component lobules.

These begin as hollow vesicles, as Meves has described them in Salamandra, in which the wall is composed of the primary spermatogonia in a single row, each enwrapped by one, or possibly two or three follicle cells. By division of the primary spermatogonia, the wall of the vesicle becomes thicker and of several layers of cells, but the descendants of each spermatogonium remain enclosed by its follicle cells and are thus separated into a group or cyst. By increase in size of the cysts, the cavity of the lobule is soon obliterated and the lobule becomes a solid mass of cells. Each secondary spermatogonium of the last generation undergoes a period of growth to form the spermatocyte, nearly doubling in size during the process. Each spermatocyte divides twice to form the spermatids which become transformed into the spermatozoa. As a result of this increase in the number and size of the component cells, the lobule, and therefore the testis as a whole, becomes greatly enlarged. During these changes the cysts are still evident, each encompassed by its follicle cells, the descendants of the cells surrounding the primary spermatogonia. When the spermatids begin their transformation, the cysts—as cysts—become disorganized, and the follicle cells assume a new function, or (probably) the old function is modified to suit the new conditions. The protoplasm increases in amount and is accumulated especially upon one side of the nucleus. In this protoplasm are inserted the heads of the maturing spermatozoa, as is the case in the organ in the higher vertebrates. When the spermatozoa are fully mature, they lose their relation to the follicle cells and accumulate in the center of the lobule ready for expulsion, while the follicle cells occupy and form the wall of the lobule. The spermatozoa finally pass from the lobule through the short tubule into the collecting duct, and thence by means of the *vasa efferentia* into the spermatic duct.

The follicle cells now undergo a degeneration and disappear, so that the lobule, as such, ceases to exist. Each lobule, therefore, has a life cycle of a year; develops, reaches maturity and degenerates, and during this time increases greatly in size, finally to shrink and disappear.

While the mass of the cells of the lobule undergo the changes of spermatogenesis, a few primary spermatogonia in the apex of the lobule (*i. e.*, near the collecting tubule) remain unchanged and persist throughout the succession of stages through which the other cells pass. When the lobule is greatly distended with the maturing spermatozoa, they become so flattened as to be rocognized with difficulty. After the spermatozoa have been extruded and the lobule has collapsed, these residual spermatogonia, surrounded by their follicle cells, again round out and present their original appearance and structure. These, by their later

multiplication, may furnish the cells for a new formation of the lobule in a succeeding season. McGregor, 99, was inclined to believe that the cells which replenish the lobule came from the cells, forming the ducts of the lobules. This seems hardly probable, since the collecting duct and its branches appear to be developed from the mesonephros, Hoffmann, 86, and not from the germinal epithelium. I believe therefore that residual spermatogonia occur in *Amphiuma* as they do in *Desmognathus,* but were not recognized as such. In other salamanders, as *Spelerpes,* regeneration of the lobule begins before the spermatozoa leave it. This does not seem to occur in *Desmognathus,* so that when the lobule is regenerated, it is as a new formation.

In *Desmognathus,* the spermatogenetic wave passes over the testis in a forward direction, that is, from the caudal to the cephalic end. There are encountered in an organ at the proper season of the year, a succession of lobules containing ripe spermatozoa, maturing spermatozoa, spermatids, spermatocytes, maturing and in division—second growing spermatocytes (of the first order), secondary spermatogonia, and finally primary spermatogonia in the more filiform cephalic end to which the organ tapers. At the opposite end the organ also becomes constricted, generally more abruptly, where it passes into the mass of interlobular connective tissue, which became more prominent when the lobules shrank and disappeared. The testis accordingly has a rather fusiform shape, tapering at both ends to a filament. The accompanying diagram indicates the succession of regions in the testis of a specimen

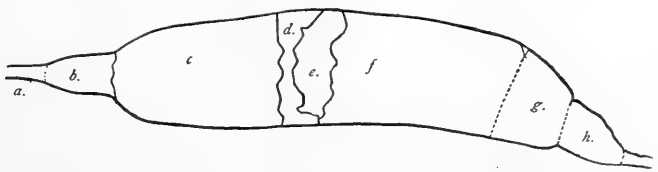

Fig. A. Diagram of a longisection of the testis of Desmognathus during July, to show the succession of regions. *a.* primary spermatogonia; *b.* secondary spermatogonia; *c.* growing spermatocytes; *d.* zone containing dividing spermatocytes of the first order and spermatocytes of the second order, resting and dividing; *e.* spermatids; *f.* transforming spermatids and immature spermatozoa; *g.* ripe spermatozoa; *h.* "degenerated" lobules.

killed in July, as seen in longisection. The shape and size of the organ, as is evident, is variable, depending on the time of year, being larger in the summer than at other seasons, and of a correspondingly different shape. Occasionally, and perhaps usually, it consists of two, three or even four enlarged portions in which the spermatozoa are

developing, these enlargements being connected by more constricted portions. The enlarged parts are identical in structure, each with a succession of stages, from the primary spermatogonia at one end to the degenerated lobules at the other. Their occurrence evidently means that there have been 2, 3 or 4 centers of growth, either primary—in the original cord of germ cells; or secondary—by the division of residual spermatogonia left when the lobule, as such, disappeared. There seems to be no absolute correlation of this condition with other structural features of the salamander, save that the presence of two or more enlargements occurs more often, in fact, quite constantly, in large animals. A similar division of the testis into "lobes" occurs in other salamanders with an elongated body, and has been noted in *Amphiuma* and *Spelerpes*. The segmented condition of the organ in Coecilians is perhaps to be associated likewise with the elongated form of body. In the European salamander, Meves, 96, and others have noted a division of the testis into lobes, whose size, shape, and possibly existence, are dependent upon the stage of development of the spermatozoa. Of these lobes, 4-5 in number, the more caudal contain ripe spermatozoa, the cephalic lobe, the cells that will form the spermatozoa, and they are so arranged that different stages follow one another in cephalo-caudal succession. In the cephalic tip, which is prolonged into a thread, the spermatogonia are located, caudad of which are the spermatocytes in successive stages of maturity or division. The lobes also possess different color according to the contents, the spermatozoa imparting a yellow-white, the cells, a transparent blue-gray. With these lobes, which are not well marked in *Desmognathus*, must not be confounded the succession of enlargements spoken of above, which are in effect independent testes.

In the spermatogenetic cycle of *Desmognathus* degenerations seem to occur quite constantly and normally. These are: (a) a degeneration of spermatozoa left over from the preceding year. This occurs in the organ in the spring, and even, it would appear, in the winter and preceding fall; (b) likewise spermatids undergo degeneration in the spring; (c) when the spermatogonia cease to undergo transformation into spermatocytes in the summer, the last cysts of spermatogonia apparently undergo a chromatolysis and solution, and the boundary between the spermatocytes which are to form spermatozoa that season and the spermatogonia remaining over until the next summer, is thus well marked. After the expulsion of the spermatozoa and the collapse of the lobule, the follicle cells degenerate and disappear. These degenerations, together with the divisions and structure of the spermatogonia

and a more detailed discussion of the structure of the Amphibian testis, the writer hopes to consider at some future time.

The Growth and Division of the Spermatocyte.

As is well known, from the spermatogonia are formed, by a process of growth, the spermatocytes of the first order. Roughly speaking, this transformation involves an increase in the size of the nucleus and the amount of cytoplasm, together with chromatic changes resulting in the formation of twelve chromosomes. The character, however, is given to the cell at the beginning of the period of growth, so that in this article the name of spermatocyte will be applied to it during this time of development, a distinction being made when necessary by applying to it the qualifying word "immature." The growth is evidently slow and takes some time for completion. In the fall and early winter the number of nearly mature spermatocytes is small. The mass of spermatozoa formed from the spermatocytes of the preceding summer, as has been said, have been largely expelled from the testis, though a few lobules usually still retain them. A few lobules likewise may contain spermatids and immature spermatozoa. During the fall, on the other hand, the spermatogonia are undergoing evidently rapid division, so that entire cysts are often found in stages of mitosis, and this process continues at a much retarded rate through the winter, to become very active again in the spring. The transformation of spermatogonia into spermatocytes, beginning in the fall, continues up to about the middle of the summer when it ceases. Divisions of the spermatocyte of the first order are but rarely found during the fall, winter and early spring, though occurring sporadically, especially in the early winter. But during late spring and early summer the divisions are abundant, continuing up to August (about), when they have nearly ceased.

A secondary spermatogonium of the last generation, small spermatogonium of Meves, 96, possesses a round or slightly oval nucleus of medium size. The chromatin is in the form of an apparent network of irregular shape and distribution. One or two small nucleoli are present. The amount of cytoplasm is small and is especially accumulated on one side surrounding the idiozome (to use the name introduced by Meves). Within the idiozome two centrosomes may usually be distinguished.

In the transformation into the spermatocyte, the network of chromatin becomes changed into a thread which is, I believe, at this time already segmented into the twelve chromosomes which are easily distinguishable a little later in their growth. The spirems are at first very fine and

intricately interwoven, the only indication of the segmentation being afforded by apparently free ends protruding on the side toward the idiozome.

Synapsis.—The beginning of the growth period of the spermatocyte is evidently a time of great importance in the process of spermatogenesis. The spermatocyte possesses chromosomes of one-half the number normally occurring in the mitoses of that species, and this pseudo-reduction has generally been located as occurring in the growth of the spermatocyte. The view, too, that the reduction in number of the chromosomes is only an apparent or pseudo-reduction, is generally accepted. That the chromosomes of the spermatocyte are bivalent and are two joined together end to end, is of course well known and also seems quite generally accepted. The terms introduced by Moore to indicate this pseudo-reduction and the corresponding period, "synapsis" and "synaptic phase," have been used in rather a confusing way. As used by Moore, 95, in his work on the spermatogenesis of Elasmobranchs, synapsis is equivalent to Rückert's, 93, *pseudo-reduction*, though Moore apparently does not assume that the chromosomes of the spermatocyte necessarily represent two joined together and therefore bivalent, which pseudo-reduction does assume. At the time that this reduction was believed to take place, there occurred in the forms studied by Moore (Scyllium and Torpedo), a peculiar contracted condition of the chromatin, causing it to be massed upon one side of the nucleus, and giving the appearance of an artifact. This Moore believed characteristic of the synaptic period and to be of general occurrence, and to this phenomenon of chromatin contraction, by a species of metonymy, the term *synapsis* has been transferred by several investigators. For this Moore himself seems partly responsible, since in a later paper, in conjunction with Farmer, 95, he uses the term synapsis as equivalent to "the contraction figure." A distinction between these two uses of synapsis seems to be necessary. The first use is evidently the correct one and is followed in this article. The use of the term in my preliminary communication upon this subject is wrong. The contracted condition of the nucleus, Moore found in the elasmobranchs investigated by him and in Amphibia (the Triton). Brauer, 93, had figured and described the massing of the chromatin upon one side of the nucleus in *Ascaris*, and Toyama, 94, figures it in the spermatogenesis of the silk worm (Moore). Paulmier, 99, describes it in insects (see also Montgomery, 00, p. 354). By botanical workers similar figures occurring at comparable points in the process of sporogenesis (the maturation period of the spore or pollen mother-cell) have been found in a large range of forms.

The observations of the investigators enumerated would indicate that the massed condition of the chromatin at the beginning of the growth period of the spore or sperm mother-cell is a natural phenomenon of general occurrence without affording any clue to its significance; on the other hand, the fact that careful investigations in spermatogenesis have been made without noting the occurrence of any such phenomenon, suggests that it is not of universal occurrence.

In *Desmognathus*, the contracted condition of the nucleus at the beginning of the growth period does occur, as was noted in my preliminary publication, and it was then assumed to be of constant occurrence in the spermatic cycle of this form. Subsequent and more careful study of the form makes this seem very doubtful. Spermatocytes are being formed during the fall, winter and spring, though growth in the winter is probably slow, and transformation ceases to take place at about the beginning of summer, as has been already stated. During this time contraction figures are found rarely, and it is not until late May or June, or in other words, in the last generations of spermatocytes, that contraction of the chromatin into a mass is general. The appearances so produced are quite similar to those found by Moore, Paulmier, Wiegand and others, and are, I think, the same phenomenon; the chromatin gathers at one side of the nucleus, leaving a space within the nuclear membrane, with which it remains connected by a few shreds of linin. At this time the chromatin is closely massed together and details of structure are difficult to make out, Fig. 18. There is no indication that anything is cast out from the nucleus at this time, as Wiegand found. With other workers, I feel confident that it is not an artifact, though no examination of fresh tissue was made. The fact that it occurs only at the end of the season of transformation at a time when the process is almost ready to stop, dissociates it, I think, from the process of "synapsis" or reduction.

At about this time there begin to appear in the last generations of spermatogonia contraction-figures which are essentially similar to those described above, in which the contraction is excessive. In these, the nucleus gathers into an apparently perfectly homogeneous round mass, the chromatin often separating from it as though "squeezed out." Such contraction-figures become abundant during July and August and are clearly, I think, degeneration changes associated with the cessation of transformation into spermatocytes, whether as cause or effect need not be considered here. Further study of this interesting phase will be undertaken in connection with the spermatogonia.

It is suggested, therefore, that the contraction-figures, instead of

being constructive and a fundamental phenomenon in the formation of the spermatocyte, may be an expression of a "running out" in the spermatogonium stock, and represent a tendency toward degeneration. We know as yet too little of the occurrence of contraction-figures in different forms to draw any general conclusions; possibly quite different phenomena may be here included. The fact of their occurrence in *Desmognathus* only at the end of the season of spermatocyte-formation is, I think, suggestive, and further knowledge of their presence in other forms from this point of view is desirable.

Growth of the Spermatocyte.—The growth of the spermatocyte has already been well described by Meves, 96, Herman, 89, and McGregor, 99, so that a detailed discussion is unnecessary. The chromatin of the spermatogonium is irregularly distributed in the nucleus in the form of an apparent network, Fig. 1. The changes in the nucleus are the following: The chromatin in the form of small granules becomes more evenly distributed in the nucleus upon the linin frame-work, which still appears to be a close reticulum with the chromatin evenly distributed. Gradually, the chromatin is concentrated in the form of a thread (or threads) connected by the linin network. It is at first hard to say whether a single thread or several (twelve) threads are so formed. Soon, however, Fig. 2, the free ends of threads are discernible projecting from the tangled mass on the side toward the idiozome. From this time on, in the growth of the spermatocyte, the chromatin threads or chromosomes shorten relatively and increase in thickness, Fig. 3; they may now be counted and are found to be twelve in number. Their free ends, typically at least, are toward the idiozome (see Montgomery, 00, p. 352), so that they form a more or less irregular horseshoe. They are made up of a succession of numerous and large chromatin granules connected together by a less chromatic substance, giving the characteristic beaded appearance shown by Hermann, and now so well known. They are not smooth but possess processes joining the linin network of the nucleus and giving to the thread (chromosome) a fanciful resemblance to a string of daisies, as has been said by others.

The establishment of the spirem from the resting nucleus of the spermatogonium, and the shortening and thickening of the chromatin threads, are but a part of one continuous process of growth, so I have considered them together rather than as belonging in part to the succeeding division. The process is one involving a considerable period of time, as judged from the large number of lobules containing growing spermatocytes in various stages of development. During this period, there is a steady increase in the size of the nucleus, as may be seen from

the figures 1-6 of Plate I. The exact method of chromatin change during the establishment of the chromosomes is rather difficult to determine. The chromatin of the spermatogonium seems to migrate out on the linin network as small granules and accumulate in lines as the spirem threads (or thread). Just in how far there is an actual migration of particles and how far it may represent a chemical change, as of the less chromatic particles to the more chromatic and the reverse (*e. g.*, as the change of oxychromatin to basichromatin of Heidenhain), giving the effect of such a migration, could not be detected.

Eisen, **00**, in his richly illustrated contribution upon the spermatogenesis of *Batrachoseps*, devotes attention largely to the finer structure of the cells. My work upon the minute details has been insufficient to render a full criticism of Eisen's views justifiable. Chromioles, chromomeres and chromosomes (his terminology) are recognized, and it seems highly probable that the chromioles do unite to form the chromosomes, though not in the fantastic way he describes. Chromoplasts were not recognized, and in the "bouquet" stage, instead of twelve "leaders" there are twenty-four, as may be seen from the transection, Fig. 4. In other words, one "wreath" does not represent two chromosomes joined by a chromoplast, but a single chromosome bent in the form of a horseshoe. Although the term "Auxocyte" was introduced by Lee as a name for the spermatogonium which becomes the spermatocyte of the first order, he fails to use it in his main work on the spermatogenesis of Helix and its introduction does not simplify nomenclature, especially when it is applied to the spermatocyte of the first order, either mature or in any stage of its growth.

It seems quite certain that there is not in this period of growth any formation of twenty-four chromosomes which then actually unite to form twelve; nor does it seem probable that a single continuous spirem is formed, which subsequently segments into the twelve chromosomes, though, as already stated, this is possible. The process appears as one of a continuous change and growth by which the distributed chromatin is gathered together in the form of twelve loops. In this case synapsis, actual or potential, would not occur as an observable fact.

But little attention has been bestowed upon the achromatic structure of the nucleus or cell body at this stage. The increase in the size of the cell body is quite large, as may be seen by comparing the figures 1-6. The idiozome, with the two centrosomes contained, shows usually three zones, which are, however, ill defined. In tissue fixed in chrom-aceto-osmic mixture there is a condensation of substance about the idiozome, as Eisen has figured it in *Batrachoseps*. This is not shown in tissue

fixed in Platino-aceto-osmic mixture, and may be due to the precipitation of proteids dissolved out when the latter fluid is used, which suggests that the idiozome acts as the nutritive center of the cell.

The two points which seem to need emphasis in discussing this stage of the spermatogenesis of *Desmognathus* are: (1) the early establishment of definite chromosomes while growth is still taking place, and (2) their polar orientation during this period of growth in relation to the idiozome and centrosomes. The early formation of the chromosomes in other Amphibia does not seem to have been very definitely recognized or noted, save in the case of *Batrachoseps* by Eisen.

The First Division.—The first indication of the division of the spermatocyte occurs in the change of position of the chromosomes. They lose the polar arrangement which they have maintained throughout their period of growth and possess no recognizable arrangement in relation to the idiozome. They do, however, exhibit a tendency to take up a superficial position beneath the nuclear membrane. The splitting of the chromatin threads follows next in close succession, Fig. 6. The details of the splitting are difficult to determine. The chromatin segments are long and still possess a bead-like structure. In them the splitting appears as a succession of clefts originating (in some cases at least) in the less chromatic part and merging at last in one continuous space. In which case, it would seem that the division did not begin with the chromatin granules. Whether or not the splitting is complete and the daughter-threads are at first separated throughout their entire length and afterwards fuse at their ends, could not be satisfactorily determined. Meves, 96, believed this was the case in *Salamandra,* and it seemed to be the case in *Desmognathus* as well, though it was hard to determine whether or not the ends seen in a section were free or cut ends. The point, however, is a minor one on our present basis of knowledge, and need not be considered further. The splitting is followed by a thickening and shortening of the double chromosomes which now are seen to be fused at their ends. In this stage they are usually distorted and twisted, assuming a variety of shapes, Figs. 7, 10. They may be twisted once, presenting the figure of an 8; they may be twisted twice; and also twisted and bent. The typical ring, O, is frequently found but by no means constantly, even in stages approaching the establishment of the equatorial plate stage.

The splitting of the chromosomes takes place before there is any perceptible change in the idiozome or the centrosomes. The latter become larger, Figs. 6, 8, 9, stain more intensely and are of vaguer outline, appearing surrounded by an umbra. They migrate apart within

the idiozome which becomes the center of radiations extending throughout the cell-body and also penetrating the idiozome itself, Fig. 9. When the centrosomes have moved apart, a delicate spindle may be seen extending between them. This spindle increases in size as the centrosomes move farther apart, and with the dissolution of the nuclear membrane, which occurs when the spindle is about half grown, penetrates the nucleus, some of the fibers becoming apparently attached to the chromosome rings as the mantle fibers. When the spindle is first formed its axis may make any angle with the nuclear membrane, Figs. 9, 10; but as it increases in size, it seems to rotate so that the axis is roughly tangential to the nuclear membrane. In some cases the spindle could be observed before the outline of the idiozome had disappeared, Fig. 10. This, together with the penetration of the idiozome by the radiations are features not observed in the other Amphibia, Meves finding in *Salamandra* that the centrosphere fragments took no part in the formation of the spindle. In *Desmognathus*, this does not seem to be the case. The chromosome loops do not seem to retreat to the opposite side of the nucleus, but rather to collect upon the side next the spindle.

The linin of the nucleus, I am sure, takes no part in the formation of the spindle, but has a different, irregular appearance, as of degeneration, and stains somewhat differently.

The chromosomes, at the stage when they are drawn upon the spindle, are irregular rings, usually much distorted; occasionally presenting the form of a Y, by partial fusion of the sides. The typical stage of the spindle formation, such as is shown in Meves' figure, in which the chromatin rings bend in response to the attachment of the mantle fibers to the ends, are of rarer occurrence in *Desmognathus*, Fig. 13. The methods employed did not reveal any difference between mantle fibers and central fibers, nor from the study of this division in *Desmognathus* did there appear to be sufficient evidence for the conclusion that the arrangement of the chromosomes on the spindle and their subsequent migration are due to the mechanical force of contraction in the mantle fibers. A careful study of the mechanics of mitosis in the spermatocyte has not been attempted, however.

The succeeding stages in the mitosis of the spermatocyte of the first order have been carefully described by Meves, Flemming and McGregor, and *Desmognathus* differs from the forms investigated by them only in apparently unimportant details. The spindle varies in shape and size though the volume of the spindle appears to remain nearly constant, the shorter spindles being broader. In *Desmognathus*, as in the other forms, the chromosomes vary in shape and actual size. The amount of fusion

of the ends of the chromosomes varies widely. In some cases the fused ends project prominently from the spindle in the equatorial plane, thus:  Such forms occur more often in the short and broad spindles, and it is believed represent an extreme expression of a tendency of the chromosomes to fuse, made possible by different "mechanical" conditions.

The disappearance of the polar radiations as the spindle develops has been commented upon by McGregor and explained by Meves as due to the absorption of their ends as the spindle grows. In *Desmognathus* the polar radiations are possibly more marked in the metaphase, but rapidly disappear in the anaphase. In the anaphase the migration of the daughter-chromosomes and their secondary fission as they pass to the poles occur in much the same way as described in other forms, Fig. 16. The secondary longitudinal fission in *Desmoganthus* is shown in Fig. 15. As the chromosomes approach the poles, they become so closely massed, Fig. 17, that the individual outlines are completely lost. This massing serves to completely mask the secondary splitting that in the early anaphase is evident. The centrosomes pass to the extreme periphery of the cell and become no longer recognizable, so that I was unable to identify them and trace them continuously to the division of the spermatocyte of the second order. A well-defined astral shield was not recognized in *Desmognathus,* nor was there any indication of a migration of the centrosomes, such as both Meves and McGregor have found in the forms studied by them. The vacuole on the polar side of the chromatin mass, which in *Salamandra* occurs under the astral shield, is, however, present.

The mid-body and the remains of the central spindle present the same characteristic appearances already well known in other forms, as may be judged from Fig. 17. No attention has been devoted to their meaning and fate.

The Second Division.—The chromosomes in the telophase of the division of the spermatocyte of the first order are closely massed in an apparently structureless mass, much contracted. When the chromatin expands to form the nucleus of the spermatocyte of the second order, the chromosomes separate from each other, and it is then seen that they occupy the same position as in the late anaphase of the previous division; their apices are turned toward the former pole of the spindle, and the branches of the V's extend back toward the opposite pole, Fig. 19. This is, of course, of typical occurrence and needs no emphasis. The chromosomes, are, however, seen to be double, so that from the apex of each, where they are united, four chromatin threads radiate out. Fig. 20

shows a stage slightly older than that illustrated in Fig. 19. A careful count at this time reveals twelve of these groups of double chromosomes. Polar views of the nucleus of the spermatocyte of the second order are shown in Figs. 20 and 21, while Fig. 22 represents a deep (equatorial) section through a nucleus, showing the chromatin threads cut across. The chromatin threads are at first long, fine, and irregular in thickness, and rough in outline, though a regular succession of chromatin granules, such as made up the chromosomes of the growing spermatocyte of the first order, is not so evident. The linin is at first scanty in amount but increases as the nucleus grows, though it never becomes relatively as abundant as in the spermatocyte of the first order. It forms a coarse mesh-work between the chromosome threads, attaching to them, and thus giving them their irregular outline. The question of the source of the linin of the spermatocyte of the second order is one to which no attention has been given in this investigation, though it is of considerable interest.

The chromosomes shorten and thicken corespondingly, thereby losing their rough outline. The groups of four chromatin threads are in this way converted into crosses or X's, which tend to lie superficially under the nuclear membrane, Figs. 23-27, as did the chromatin rings in the spermatocyte of the first order, and so lose their original polar orientation. At about the time of the dissolution of the nuclear membrane, the X's separate into their component V's which become involved in the spindle, Fig. 29. Their distribution at the time they are first formed is apparently without order or system, so that the equatorial plate stage is one of loose formation. Typically, when the cross is dissolved, the two component V's become applied to each other, and thus are drawn into the equatorial plate in pairs, Fig. 29. This, however, does not seem to be always the case, so that often the V's are separated from each other and promiscuously scattered.

The remaining stages in the mitosis of the spermatocyte of the second order have been well described by other workers, Flemming, Meves, McGregor, and need not be repeated here. The equatorial plate, anaphase and telophase are shown in Figs. 30, 31 and 32, and from them one may see that the processes are essentially identical with those described long ago by Flemming in *Salamandra*. The chromosomes become closely massed as they pass to the pole, as was the case in the spermatocyte of the first order, Fig. 32. They become separated again in the dispirem stage, and are once more apparent as V's, twelve in number, with their apices toward the pole of the cell, Fig. 33. These soon lose their form and give rise to a fine reticulum characteristic of

the spermatid. Fig. 34 is a transection of the expanding nucleus of the spermatid, showing the arms of the V's cut across, while Fig. 35 shows the fully formed spermatid. That the nucleus of the spermatid undergoes a slight enlargement before the period of transformation into the spermatozoon is evident.

The determination of the exact method in which the spindle is established in the spermatocyte of the second order has been attended with considerable difficulty, and despite careful work with many specimens well fixed and stained, it has been impossible to arrive at an absolute decision. The spindle is established from a stage similar to that shown in Fig. 27. One centrosome lies at an extreme side of the cell-body directly under the cell-membrane, and from it there extends a radiation of fibers. The other centrosome lies close to the nuclear membrane some distance from the first, and it likewise is the center of radiations. The spindle seems to be formed by the fusion of these two sets of radiations. The earlier history of the formation of the achromatic figure is not so clear. The identity of the centrosome at the end of the first mitosis is lost, as has already been stated, so that centrosomic continuity between the first and second divisions has not been established; nor has it been shown that the two centrosomes of the second division were derived from a single centrosome, which presumably would have been one of the daughter-centrosomes of the previous division. Fig. 25 suggests that this is the case, and that my failure to trace them has been due to the fact that one of them moved close to the nuclear membrane and on that account and because of the absence of well-marked radiations became in most cases indistinguishable. The entire achromatic figure, in comparison with the one of the previous division, is weak. A centrosphere is lacking, radiations are not well marked, and the astral shield at the end of the division is not developed.

From an examination of the Amphibian literature, it appears that there are no figures showing the development of the spindle in the second division. McGregor failed to trace the continuity of the centrosome in the spermatocyte divisions of *Amphiuma*, though Meves, in his description, leaves no doubt as to his interpretation of centrosomic continuity. Eisen, in *Batrachoseps*, describes and figures the spindle in the second division as formed by a fusion of two sets of radiations (fiber-cones) resembling somewhat the method described above as the one believed to occur in *Desmognathus*. In his work, new-formation of centrosomes (archosomes) is assumed to occur. His fiber-cone resembles closely one of the centrosomes in *Desmognathus*, surrounded by its radiations, and undoubtedly the two are the same.

The life of the spermatocyte of the second order must be short, very short as compared with the growth period of the spermatocyte of the first order and the transformation period of the spermatid, since never at any time do more than a few lobules contain spermatocytes of the second order. In size these are markedly smaller than the spermatocytes of the first order, and during their existence do not show an appreciable increase in size. The chromosomes remain distinct throughout, never losing their individuality, though a nuclear membrane is formed with a development of linin.

General Considerations.

In the brief discussion just given of the divisions of the spermatocyte, questions of their interpretation as mitoses have been entirely ignored. While sufficient attention has not been devoted to the study of the "mechanics" of the divisions, a word may be vouchsafed on certain points. Any contribution offering correct or suggestive interpretations in this most difficult field must, at the present time, be the result of comparative work, and that I have not done in this case. My aim has been, therefore, to keep the analysis of cell and nuclear structure as simple as possible, ensuring only its being sufficient for the purposes of this paper. Eisen's more elaborate analyses of granosphere, plasmosphere, hyalosphere and cone-fibers, were not found applicable in *Desmognathus;* they are felt to be premature. The purely mechanical interpretation of the processes of division given by Eisen (and others), I cannot consider at all satisfactory, nor can his statement that "the mitosis of the cells of the testis of *Batrachoseps* is the result of *two independent parallel processes coöperating only at certain points,*" receive my confirmation from the study of the same divisions in *Desmognathus.* In the maturing spermatocyte of the first order, the arrangement of the developing chromosomes with their free ends toward the idiozome, suggests an interaction of the two during this period, and the idiozome as the metabolic center of the cell-body. As has been said, if Flemming's fluid is employed instead of Hermann's fluid, there is revealed a massing of substance about the idiozome not indicated by the other fixer—quite possibly soluble proteids. The loss of orientation when growth is attained, the splitting of the chromosomes followed by the changes in the idiozome point, I believe, to an intimate interrelation of the two sides of the phenomena. Meves, McGregor and Eisen have assumed that the arrangement of the chromosomes on the spindle and their subsequent migration are due to a force of contractility in the

mantle fibers, which seems to me quite inadequate as an explanation from the conditions in *Desmognathus*.

The chromatin changes in *Desmognathus*, considered by themselves and briefly stated, are as follows: The chromosomes, twelve in number, (presumably) one-half that of the somatic mitoses, develop as horse-shoe-shaped threads, the free ends pointing toward the idiozome. These split longitudinally and incompletely, the ends being fused and open out to form rings in the manner typical in Amphibia. In the anaphase, however, the fused ends separate and daughter-V's are formed. As these pass to the poles, a secondary splitting takes place, which (presumably) masked in the late anaphase, reappears in the expansion of the nucleus of the spermatocyte of the second order, when the chromosomes are found to be united at their apices. These united V's shorten and thicken to form crosses and X's, which in the metaphase separate into the two component V's. In my preliminary paper was set forth a discussion of the chromatin changes in view of the possibility of a "reducing" division in *Desmognathus*, and a portion of what was then said may be repeated here. It was there pointed out that the formation of the crosses in the spermatocyte of the second order and their subsequent solution into V's introduced the possibility of a reducing division, since it was not possible to determine in what plane the separation into V's took place. Granted the V's represent bivalent chromosomes, the result of the splitting and cross formation gives  If the separation into V's simply completes the longitudinal splitting, we have  and no reducing division; if, however, it takes place at right angles the resulting V's are and the division is a

"reducing" divisions. To quote from that article, 99: "If the second division in *Desmognathus* is to be looked upon as a reducing division, it may be considered in two ways. The original union of the chromosomes, after two longitudinal splittings of the united chromosomes, is now dissolved and a new union between the daughter-chromosomes established; or, from the standpoint of the more typical mode of reduction by tetrad formation with longitudinal and transverse divisions, there would occur in *Desmognathus*, a reduction in number to one-half, a longitudinal (equation) division, which, however, is not completed, and is prevented from being completed, by the second division, which is transverse. Shorten the interval elapsing between the first and the second divisions, and (possibly thereby) eliminate the second longitu-

dinal splitting, and the process is reduced to the typical form." Such a transverse division is not believed to occur in *Desmognathus*, however, and the above is written simply to present all the possibilities of interpretation. If we compare other Amphibia we find that the occurrence of a fusion between the apices of the daughter-V's with X-formation does not exist as far as reported, save perhaps in the oögenesis of the Triton, as investigated by Carnoy and Le Brun, 98. Flemming, 87, in his work, indeed, did not recognize that the longitudinal splitting of the chromosomes of the secondary spermatocyte took place early (in the previous cell generation), nor that the splitting of the daughter-chromosomes in the anaphase of his heterotypic mitosis was the precocious splitting for a second, following division; though he recognized its importance and normal occurrence, he confessed ignorance as to its significance. Meves, 96, leaves no doubt that this second precocious splitting becomes completed in the spermatocyte of the second order as the longitudinal division of the chromosomes of that cell division; his words are: "Die zweite homoötypisch verlaufende Reifungstheilung schliesst sich an die erste heterotypische an, ohne dass ein eigentliches Ruhestadium des Kerns durchlaufen wurde, sondern dieser tritt aus dem Dispiremstadium von neuem in Mitose. Indem sich die chromatischen Fäden auflockern, wird zunächst die im Dyaster der heterotypen Form aufgetretene Längsspaltung welche wahrend des folgenden Dispiremstadiums undeutlich geworden war, von neuem sichtbar" (p. 61). McGregor,[4] too, inclines to the same result in *Amphiuma*, while Eisen does not refer to the steps in sufficient detail, stating simply that both divisions in *Batrachoseps* are equation divisions. Carnoy and Le Brun, 98, in their work on the oögenesis of the Tritons, agree with the other workers on Amphibia in that they find both divisions are longitudinal. Their results are unique in several particulars. Their figures show the occurrence in the oögenesis of Triton, of X's entirely similar in appearance to the structures in *Desmognathus*, though it does not appear that they are daughter-V's united at their apices and formed by an incompleted longitudinal splitting. Their figures illustrating the second longitudinal splitting do not appeal to me as satisfactory, and perhaps permit of a different interpretation. The two divisions in the oögenesis of the Tritons follow each other more rapidly, so that a species of tetrad-

[4] "The chromatin emerges from the spirem in the form of twelve V's longitudinally split, which are probably identical with those of the anaphase of the preceding division, though this cannot be stated with absolute certainty, for it is impossible to discover exactly how the new double V's arise from the spirem." McGregor, 99, p. 80.

formation occurs. The second (axial) splitting may be postponed and occur in the anaphase as the chromosomes are passing to the poles. The figures strongly suggest that there is a close resemblance to the divisions in the spermatogenesis of *Desmognathus,* modified by the more rapid succession of the division in the polar-body formation.

From these comparisons there seems little doubt as to the interpretation of the second division in *Desmognathus* as a longitudinal splitting, nor as to its being the persistent longitudinal splitting which occurred in the anaphase of the first division. The second splitting in Flemming's heterotypic mitosis is clearly, then, the precocious division of the chromosomes for the succeeding division, and should not be considered an essential character of heterotypic division, since it would not necessarily occur. I believe that *Amphiuma* agrees with *Desmognathus* in this respect. The interpretations of Carnoy and Le Brun are unique, and cannot be reconciled with my own findings.

It is not necessary here to refer in detail to the influence Weismann's theory of the germ-plasm has had upon spermatogenesis work. Practically the only detailed work that had appeared prior to his first publication, in 1887, touching on the question of a reduction was Flemming's classical paper upon the divisions of the spermatocyte in *Salamandra maculosa.* Under the stimulating influence of Weismann's essay, paper after paper appeared—by Henking, vom Rath, Rückert, Häcker and others—some of which seemed to bring wonderful proof of the correctness of his prophecy, while in other cases, as those of Brauer, Boveri, Moore, Meves, etc., the results were contradictory.

Owing largely to Weismann's theory and its apparent confirmation, there has been a powerful impetus given to the work in oögenesis, spermatogenesis, fertilization and cleavage, and from the standpoint of his brilliant theory new possibilities of interpretation of the phenomena of development have been brought out in testing its accuracy. In so far as it has led to these results, much could be said of the beneficial influence "Weismannism" has had in biology. On the other hand, in the investigation of oögenesis and spermatogenesis, the study of the phenomena has been made too largely a search for the occurrence of tetrad-formation and reducing divisions. An unproved theory, a speculation, highly suggestive and stimulating, but altogether hypothetical and not admitting of even partial proof, has been made the basis of the work, and it has diverted attention from other points of view that would have given a more normal, though perhaps not so rapid, development of this field of work.

A truer basis upon which an interpretation of the phenomena of

spermatogenesis should be attempted is that of mitosis. The two "reducing" divisions are mitoses with certain peculiarities and should be considered simply as such and investigated from that standpoint. Any explanation of oögenesis or spermatogenesis must be first of all an explanation of cell-divisions. I do not mean that this has not been done by many workers on spermatogenesis; and full appreciation is felt of the excellence of the work of those employing spermatogenesis divisions for the investigation of mitosis. Flemming's classical paper in 1887, with its recognition of the divergent types of mitosis, uninfluenced as it was by theoretical interpretations, seems to me to represent a much more healthy attitude than do many of the later contributions. Occasionally the influence of theory has been responsible for evident errors of interpretation, as, in Amphibia, vom Rath's work on the spermatogenesis of *Salamandra*.

As is well known, in several groups, by repeated and confirmatory investigations, the absence of "reducing" divisions has been shown, and this is especially evident in Amphibia, *Ascaris*, and *Lilium*. In Amphibia, Flemming, Meves, McGregor, Carnoy et Le Brun, Eisen and myself have furnished strong demonstration. *Ascaris megalocephala* has been tested by Boveri, Brauer and Hertwig. Among plants, small doubt may be felt about the divisions in the *Liliaceæ* from the work of Strassburger, Guignard, Mottier, Sargent and Dixon. A single well-authenticated case of the absence of transverse divisions seems to me to be fatal to the theory of a qualitative reduction, and warrants its rejection as a working hypothesis. In its abstract form, it is a theory that cannot be disproved, although as reconstructed it cannot offer a more suitable basis for interpretation. While in certain forms both divisions of the spermatocyte have been shown to be longitudinal,[5] in other groups I think it may be considered fairly well proved, that one of the divisions is as certainly transverse. Carnoy and Le Brun, it seems to me, go too far in doubting correctness of observation in the finding of transverse divisions. In Insects and Copepods, certainly, the concordance of results permits but one interpretation—that one of the divisions is transverse. Both conditions must be harmonized, then, in any theory of spermatogenesis, and this the Weismann theory does not do.

If we view the divisions of the spermatocyte from the standpoint of

[5] In a recent paper by King on the oögenesis of Bufo, the conclusion reached is that there both divisions are equation divisions, the "splitting" in the first maturation division taking place very early.

mitosis, three features are to be noted; the first of these, is the rapidity with which the divisions follow each other, without an intervening interval of rest and growth. The effect of this is, theoretically, a reduction in the size of the nucleus of the grand-daughter cells one-half. In ordinary mitoses, the nucleus, n, increases by growth to 2n, divides so that the daughter-nuclei represent n: by growth each of these increases to 2n, to be reduced in the ensuing division to n, and so on. Omit one of the periods of growth so that the second division follows immediately after the first, and the nuclei in the daughter-cells of the second division are reduced to $\frac{1}{2}$n. A quantitative reduction of the nuclear matter to one-half is accomplished as an inevitable result of the two rapidly following divisions. The difference in relative size of the cells of the generations of the spermatocyte divisions may be easily seen by comparing the figures, after reducing those of Plates III and IV, as directed. The spermatogonium has a nucleus with a diameter of, say, 25n, the nucleus of the mature spermatocyte measures 32n, that of the secondary spermatocyte has a diameter of 25n, while the spermatid has, as the corresponding measurement, 18n. This, of course, gives but the grossest idea of the size differences of the cells and nuclei. The size of the nucleus depends in part upon the growth period it has enjoyed, and this, in turn, must depend upon numerous factors, among them the metabolic interrelation of nucleus and cell-body, so that, considered quantitatively, the size of the nucleus is largely relative and variable. In the divisions of the spermatogonia there is such a variation in the size of the nuclei that it would be very difficult to estimate the size relative to the original embryonic nuclei. The primary spermatogonia possess large nuclei; these undergo rapid division and there is a decrease in the size accordingly. The period of growth of the spermatocyte again increases the size of the nucleus, restoring it—may we assume?—to the original size before a division. Only in case we assume that the quantity of nuclear matter in embryonic cells remains approximately constant, and that the mature spermatocyte has a nucleus as large as that of an embryonic cell before a division, is it safe to state that the divisions of the spermatocyte accomplish a quantitative reduction to (approximately) one-half.

The second point that seems well established is that in the spermatocyte mitoses the chromosomes appear in one-half the number that has been found in the ordinary tissue (and embryonic) mitoses in the respective forms. The significance that this seems to possess is the prevention of the doubling of the chromosomes and the maintenance of their numerical constancy in the species. It is, therefore, prophetic,

anticipatory. It has theoretical bearings on the meaning of the constancy of the number of the chromosomes and their individuality. Great as the evidence is, my inclination is to regard the generalizations as to the importance of a constancy in number of the chromosomes and the adherence to that number in the mitoses of different cells in the organism, as yet unsafe.

The same doubt may be applied to the question of the individuality of the chromosomes, for which the evidence is not as strong. On the basis of the individuality of the chromosomes rests the interpretation of the reduction in number of the chromosomes as to a synapsis or a joining together in pairs, so that each is bivalent. This view may be purely hypothetical and unobserved, as in Moore's work, or based on actual observation, as in Montgomery's. In *Desmognathus* there is no evidence that the chromosomes of the spermatocyte are bivalent; nor in other Amphibia do we find evidence reported, save perhaps by Eisen in *Batrachoseps* where the chromoplasts may be interpreted as joining or separating single chromosomes. In that case, however, the number of chromosomes is one-half what it should be in that form.

The third general feature that attracts attention is the existence of the peculiar chromosome-forms that characterize the spermatocyte divisions, among which may be included, tetrads, ring-forms, X-forms, Y-forms, and V-forms different from the V-shaped chromosomes of ordinary mitoses. It seems likely that the differences which exist between the spermatocyte divisions in different forms is due to minor modifications of the procedure, and that they are not intrinsic, so that if the modifying causes could be recognized, the variations could be more easily understood.

As a peculiarity of the spermatocyte mitoses has been mentioned the lack of a period of growth between them. The rapidity with which the second division follows the first in spermatogenesis seems to vary. Perhaps Scyllium, according to Moore's account, presents, among observed forms, the most complete resting stage between the first and second divisions. Here a complete resting period intervenes, with new formation of chromosomes in the second division. In Mammals likewise there is apparently a new-formation of the chromosomes after a resting period, Lenhossek, 96.

In Amphibia there is encountered a step toward the shortening of the interval. In *Salamandra*, according to Meves' account, a true dispirem, not to say a reticulum, does not seem to exist, and in *Desmognathus* the chromosomes remain distinct, though they become irregular and thread-like. The second splitting, furthermore (if we may accept this inter-

pretation), has moved forward from the second spermatocyte into the anaphase of the primary spermatocyte. In the oögenesis of the Tritons, Carnoy et Le Brun, as already stated, find that the second splitting follows the first so rapidly that by the two splittings, a chromatin ring (tetrad) is formed, though sometimes the second splitting appears in the anaphase of the first division. In all these cases the chromatin division is by longitudinal splittings not (in this respect) markedly different from those of ordinary mitoses, but becoming less typical as the resting period is shortened and the chromatin fission is shifted toward the first mitosis. In all, however, the intervening period of growth is inadequate to restore the chromosomes to their former size, and the chromosomes of the second division are markedly smaller than those of the first, presumably approximately one-half their size, as commented on by Moore, Lenhossek, Meves, Carnoy et Le Brun.

From the Amphibia, it is but a step to the condition described by Boveri in *Ascaris*, where the second chromosome division, occurring as a longitudinal fission before the first mitosis, forms tetrads by a double longitudinal splitting, which Boveri himself interpreted as a precocious splitting due to or associated with the lack of nuclear reconstruction between the two divisions.

Why it is that, in what is generally regarded as the more usual method of tetrad formation, the chromatin preparation for the two divisions is accomplished by the first longitudinal splittings being (in the typical case) followed by a transverse splitting as the second division instead of another longitudinal one, is, of course, on the basis of our present knowledge, entirely inexplicable. The nature of the changes that go on in the cell and induce spirem and chromosome formation and longitudinal fission is equally unknown, and the explanation of why in certain forms and certain mitoses the daughter-chromosomes are formed by a transverse instead of a longitudinal separation, must be wrapped up with the explanation of the former; in other words, the exception must be explained with the rule. Any discussion of the side of mitosis, to which this leads, is beyond the scope and ambition of this article, but the interpretation of the transverse division is to be sought in that portion of mitosis phenomena in general. The fact, however, that in the same divisions, in different forms, the separation occurs in different ways, indicates that the plane of fission is not the determining factor, or intrinsically important, but is itself determined by other factors. Forms in which tetrad formation is accomplished by means of at least one transverse division are, I believe, all forms in which

the second division of the cell follows the first without any resting period.

A second point of view from which the chromosome forms may be considered is that of their manifest tendency to fuse. Thus, ring-formation typically occurs by the fusion or incomplete fission—which is in effect the same—of the ends of the chromosomes. Almost every conceivable variety and modification of the typical ring is to be met with depending on the region and extent of the fusion; Y-forms, T-forms, solid rods, crosses and V's, have all been found. The ring-form is the type and from it all others of these varieties may be derived by the increase of the fused area. Rings and their modifications are found almost constantly in the large majority of forms, with or without tetrad formation, in the first division of the spermatocyte. The more exceptional forms derived from it, while rarer, yet occur also in the first division of the spermatocyte. Thus, Griffin found in *Thalassema*, Y-forms, rod-forms, and X-forms; van der Stricht found in *Thysanozoon* rod-forms with gradations of fusion modified from the ring-form; v. Klinckowström, ring-forms, cross-forms, and rod-forms in the Planarian, *Prostheceræus*. Many other instances of excessive fusion in the chromosomes of the spermatocyte of the first order might be given in both animals and plants; *e. g.*, Belajeff, 92, Atkinson, 99.

Fusion of the daughter-chromosomes in their middle instead of at the end produces the characteristic X- and +-forms, when they are more or less V-shaped, and as the fusion extends out on the legs of the V-, Y- and T-forms as well. These have also been found in the divisions of the spermatocyte in a number of forms, and usually in the division of the spermatocyte of the second order rather than in the previous mitosis. Thus, by van der Stricht in *Thysanozoon;* by v. Klinckowström in *Prostheceræus;* in Cyclops by Haecker; by myself in *Desmognathus*. Among plants crosses were found in *Larix* by Belajeff, in *Hemerocallis* by Juel. In *Allium*, Ishikaua reports the X-formation by fusion of the daughter-chromosomes at their apices in the first division; while in the second division, the chromosomes unite by their ends to form rings, there being thus a reversal of the condition found by me in *Desmognathus*, which is perhaps more typical. Crosses may, therefore, be formed as a modification of the end fusion (ring-formation), or by the center (apex) fusion of the daughter-chromosomes. These two forms—O and X—seem to be the types from which other varieties of chromosome form in the spermatocyte are derived.

In tetrad-formation, based on the ring-formation, the tendency toward

fusion of the daughter-chromosomes *inter se* is not so marked, though in certain cases it seems to be exhibited; *e. g.*, in *Anasa*, Paulmier, 99.

The significance of this tendency toward fusion and its cause are, of course, obscure. Possibly it is due in part to a more labile condition of the chromatin in the spermatocyte, which would cause the chromosomes to run together and round off whenever other forces permitted and in a corresponding manner and degree. This marked lability is indicated by a number of facts that appeal to one in studying the divisions in a particular form, as well as in examining the published figures of the conditions in other forms: the strong tendency of the chromosomes to mass together as they approach the poles of the spindle in the anaphase; the great irregularity of the forms of chromosomes presented; and the readiness with which they change shape. Typical tetrad-formation itself may be in part the expression of this same tendency of the chromatin to round off, due to a more fluid consistency.

Whatever the factors upon which the ring and cross formations depend, it is my belief that they cannot be interpreted apart from the entire problem of cell division. I fully appreciate that no real explanation is offered in what has been written above; nor is it meant as a criticism of the excellent work done upon spermatogenesis and oögenesis. Those investigations in which the spermatocyte divisions have been studied as mitoses are also recognized. I simply present my view as to the standpoint from which the phenomena of spermatogenesis should be considered in order that a firmer basis be given for interpretation; that view is,—that the divisions be studied as such, and the case and effect of the omission of the second growth period be sought; that the chromatin changes be considered from the standpoint of chromatin changes elsewhere and as a part of the entire phenomenon of cell division.

If we return again to a consideration of *Desmognathus*, we find in the first mitosis, ring-formation, usually quite irregular and variable; in the second division, cross-formation. In the first division it is the *ends* of the chromosomes that show the fusion more strongly, and rings result; in the second division their *middle points* (the apices of the V's) fuse and X's result, the behavior of the daughter-chromosomes of the two divisions in this respect being antithetic, a condition that may have significance and which occurs also in other forms (v. Klinckowström, van der Stricht, Ishikaua). If we consider the chromosomes in the two spermatocytes in their relation to the pole of the cell (centrosome), in the growing spermatocyte, the chromosomes form loops with their ends toward the centrosomes; in the spermatocyte of the second order, the

apices of the V's (their middle points) are toward the centrosome (?), and in each case the portion toward the pole of the cell becomes fused. This is offered without comment as worth consideration, in the firm conviction that the chromatin changes cannot be explained in themselves.

Assuming that the chromosomes of the spermatocyte are bivalent (of which, as said, there is not evidence in *Desmognathus*), then it is that in the first mitosis *one* end of the united chromosomes fuses; in the second, the *opposite* end, suggesting a polarity in the chromosomes themselves, which, indeed, their bivalence itself might be interpreted as indicating.

Summary.

1. The "contraction figures" in the nucleus of the growing spermatocyte do not occur constantly in *Desmognathus*.

2. The chromosomes of the spermatocyte are twelve in number, and in their growth are horseshoe-shaped with the ends toward the idiozome and centrosomes, suggesting a polarity of the cell at this stage.

3. Synapsis was not observed in the formation of the chromosomes of the spermatocyte.

4. The first division of the spermatocyte is heterotypic, with ring-formation by incomplete splitting.

5. The second splitting of the chromosomes in the first division is believed to be the precocious fission of the second division.

6. The daughter-chromosomes of the second spermatocyte remain fused together at their apices to form X's.

7. Both divisions of the spermatocyte are believed to be equation divisions, and no qualitative reduction takes place.

8. The spindle in the second division is believed to be formed by the fusion of two sets of radiations.

9. The first and second divisions of the spermatocyte have certain similarities and differences that are suggestive. A comparison with other forms is given.

10. The structure of the testis, spermatogenetic cycle, and the life cycle of the lobules are discussed.

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LITERATURE CITED.

Atkinson, G. F., 99.—Studies on reduction in plants. I. Reducing division in Arisaema triphyllum by ring and tetrad-formation during sporogenesis. II. Reducing division of the chromosomes in Trillium grandiflorum during sporogenesis. Bot. Gazette, Vol. XXVIII, pp. 1-26.

Belajeff, W., 92.—Ueber die Karyokinesis in den Pollenmutterzellen bei Larix und Fritillaria. Sitzb. Warsch. Naturf. Ges.

Bidder, F. H., 46.—Vergleichend Anatomische und Histologishe Untersuchungen über die männliche Geschlechts und Harnsorgane der nakten Amphibien. Dorpat., 1846.

Boveri, Th., 90.—Zell Studien, Heft. 3. Ueber das Verhalten den chromatischen Kernsubstanz bei der Bildung der Richtungskörper und bei der Befruchtung. Jen. Zeitschr. f. Naturw., Bd. XXIV, 1890.

Brauer, A., 93.—Zur Kenntniss der Spermatogenese von Ascaris megalocephala. Arch. f. mikr. Anat., Vol. XLII, pp. 153-213.

Carnoy, J. B. et Le Brun, H, 98.—La vesicule germinative et les globules polaires chez les batraciens. La Cellule, Vol. XVI.

Dixon, H. H., 94.—Annals of Botany, Vol. VIII.

Eisen, Gustav, 00.—The spermatogenesis of Batrachoseps. Polymorphous Spermatogonia, Auxocytes and Spermatocytes. Journ. of Morph., Vol. XVII, pp. 3-117.

Flemming, W., 87, 91.—Neue Beiträge zur Kenntniss der Zelle. Arch. f. mikr. Anat. Theil I, Vol. XXIX; Theil II, Vol. XXXVII.

McGregor, J. H., 99.—The Spermatogenesis of Amphiuma. Journ. of Morph., Vol. XV, Supplement, pp. 57-104.

Griffin, B. B., 99.—Studies on the maturation, fertilization and cleavage of Thalassema and Zirphaea. Journ. of Morph., Vol. XV, pp. 583-634.

Guignard, L., 91.—Nouvelles Études sur la Fécondation. Ann. d. sci. nat. Bot., T. XIV, 1891.

Haecker, V., 99.—Praxis und Theorie der Zellen und Befruchtungslehre. Jena, 1899.

Henking, H., 91.—Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten. III Specielles und Allgemeines. Zeitschr. f. wiss. Zool., Vol. LIV; also Vol. XLIX; LI.

Hermann, F., 89.—Beiträge zur Histologie des Hodens. Arch. f. mikr. Anat., Vol. XXXIV.

Hertwig, O., 90.—Arch. f. mikr. Anat., Vol. XXXVI.

Hoffmann, C. K., 78.—Bronns Klassen und Ordnungen des Thierreiches. Bd. VI, 2te Abth. Amphibien.

Hoffmann, C. K., 86.—Zur Entwicklungsgeschichte der Urogenitalorgane bei den Anamnia. Zeitschr. f. wiss. Zool., Vol. XLIV, 1886, pp. 570-643.

Ishikaua, M., 97.—Studies of Reproductive Elements. III. Die Entwicklung der Pollenkörper von Allium fistulosum L., ein Beitrag zur chromosomenreduction im Pflanzenreiche. Journ. Coll. Sci., Tokyo, X, 2.

Jordan, E. O., 91.—The Spermatophores of Diemyctylus. Journ. of Morph., Vol. V, pp. 263-270.

Juel, H. O., 97.—Die Kerntheilungen in den Pollenmutterzellen. Jahrb. d. wiss. Bot., Vol. XXX.

Kingsbury, B. F., 95.—The Spermatheca and Methods of Fertilization in some American Newts and Salamanders. Proc. Am. Micr. Soc., Vol. XVII, 1895, pp. 261-304.

Kingsbury, B. F., 99.—The Reducing Divisions in the Spermatogenesis of Desmognathus fusca. Zool. Bulletin, Vol. II.

Klinckowström, A. V., 97.—Beiträge zur Kenntniss der Eireife und Befruchtung bei Prostheceraeus. Arch. f. mikr. Anat., Vol. XLVIII.

Lee, A. B., 97.—Les Cinèses Spermatogénétiques chez l'Helix pomatia. La Cellule, Vol. XIII, pp. 199-278, 1897.

Lenhossek, M. von, 96.—Untersuchungen über Spermatogenese. Arch. f. mikr. Anat., Vol. LI.

Leydig. Untersuchungen über Fischen und Reptilien.

Meves, F., 96.—Ueber die Entwicklung der männlichen Geschlechtszellen von Salamandra maculosa. Arch. f. mikr. Anat., Vol. XLVIII.

Moore, J. E. S., 95.—On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs. Quart. Journ. Mikr. Sci., Vol. XXXVIII, 1895.

Moore, J. E. S. and Farmer, J. B., 95.—Essential Similarity of the chromosome reduction in Animals and Plants.—Annals of Botany, Vol. IX, p. 435, 1895.

Mottier, D. M., 97.—Beiträge zur Kenntniss der Kerntheilungen in den Pollenmutterzellen. Jahrb. d. wiss. Bot., Vol. XXX, 1897.

Montgomery, Th. H., 98.—The Spermatogenesis of Pentatoma up to the formation of the Spermatid. Zool. Jahrb., Vol. XII; Abt. f. Anat. u. ontogenie.

Montgomery, Th. H., 00.—The Spermatogenesis of Peripatus (Peripatopsis) balfouri up to the Formation of the Spermatid. Zoolog. Jahrbüchern: Abth. f. Anat. und Ontogenie der Thiere, Vol. XIV, pp. 277-368, 1900.

Paulmier, 99.—The Spermatogenesis of Anasa tristis. Anat. Anz., Vol. XIV; Journ. of Morph., Vol. XV, suppl., pp. 223-272.

Vom Rath, O., 93.—Zur Kenntniss der Spermatogenese von Salamandra maculosa. Zeitschr. f. wiss. Zool., Vol. LVII.

Vom Rath, O., 92.—Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris Latr. Mit besonderer Berücksichtigung der Frage der Reductionstheilung. Arch. f. mikr. Anat., Vol. XL, p. 102.

Ritter, W. E., and Miller, Loye, 99.—A contribution to the life-history of Autodax lugubris, Hallow, a Californian Salamander. Am. Nat., Vol. XXXIII, 1899, pp. 691-704.

Rückert, J., 93.—Die Chromatinreduction der Chromosomenzahl im Entwicklungsgang der Organismen. Merkel & Bonnet, Ergebnisse, Vol. III.

Sargent, Ethel, 96, 97.—The formation of the Sexual Nuclei in Lilium Martagon. Annals of Bot., Vol. X; Vol. XI.

Sherwood, W. L., 95.—The Salamanders found in the Vicinity of New York City, with notes on extra-limital or allied Species. Proc. Linnean Soc. of New York, No. 7, pp. 21-37.

Spengel, 76.—Das Urogenitalsystem der Amphibien. Arbeiten des zoolog.-zootom. Instituts in Würzburg, Vol. III, Heft. 1, 1876.

La Valette, St. George, 76.—Ueber die Genese der Samenkörper. Die Spermatogenese bei den Amphibien. Arch. f. mikr. Anat., Vol. XII.

Strassburger, E., u. Mottier, 97.—Ueber den zweiten Theilungsschritt in Pollenmutterzellen. Ber. d. Deutsch. Bot. Ges., Vol. XV, nr. 6.

van der Stricht, 98.—Contribution à l'étude, de la forme, de la structure, et de la division du noyau. Bull. de l'acad. de Belgique, Ser. 3, T. XXIX, 1898.

Toyama, 94.—On the Spermatogenesis of the Silk-worm. Bull. Agr. Coll. Imp. Univ., Tokio, Vol. II, No. 3.

Weismann, A., 87.—Ueber die Zahl der Richtungskörper und über ihre Bedeutung für die Vererbung. Jena, 1887.

Weismann, A., 93.—The Germ-plasm. New York.

Wilder, H. H., 99.—Desmognathus fusca (Rafinesque) and Spelerpes bilineatus (Green). Am. Nat., Vol. XXXIII.

Wilson, E. B., 00.—The Cell in Development and Inheritance. The MacMillan Co., New York, 1900.

von Wittich, 53.—Beiträge zur Morphologische und Histologische Entwicklung der Harn und Geschlechtswerkzeuge der nakten Amphibien. Zeitschr. f. wiss. Zool., Bd. IV, 1853.

Zeller, Ernest, 90.—Ueber die Befruchtung bei den Urodelen. Zeitschr. f. wiss. Zool., Vol. XLIX, pp. 583-602, 1890.

Explanation of Plates.

All the figures of the four plates are drawn at the same magnification with a Leitz microscope, draw-tube in, Leitz 1/16 in. immersion objective, and Leitz No. 4 Ocular. All figures on plates III and IV are reduced only ½ the extent of plates I and II. ⅓ was taken off any diameter of figures on plates I and II. Only ⅙ taken off any diameter of figures on plates III and IV. Hence take ⅕ more off any diameter of a figure on last two plates, to compare size with a figure on first two plates. All were drawn from longitudinal sections of *Desmognathus* testes, fixed in Hermann's fluid (except Fig. 7, where the fixer was Flemming's chrom-aceto-osmic mixture, strong formula) and stained with Heidenhain's iron hematoxylin. The sections from which they were drawn, were paraffin sections, 10 and 7 μ thick.

PLATES I AND II.

Division of Spermatocyte I.

Fig. 1. Secondary spermatogonium of the last generation, showing as yet no indication of the beginning of the growth period.

Fig. 2. Spermatocyte soon after the beginning of the growth has become evident. The chromatin is becoming arranged in the form of threads. A nucleolus is shown.

Fig. 3. Spermatocyte, later stage of growth; spirems larger and better defined. Indication of the chromosome threads with their free ends towards the idiozome.


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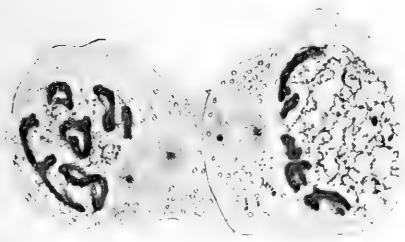

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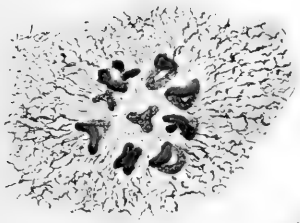

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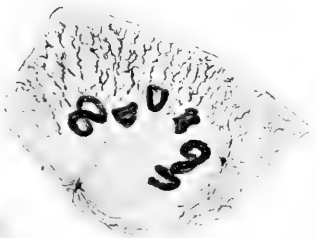

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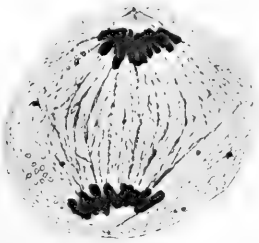

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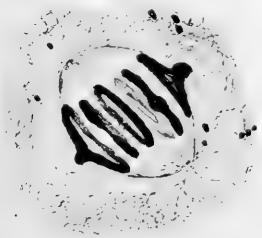

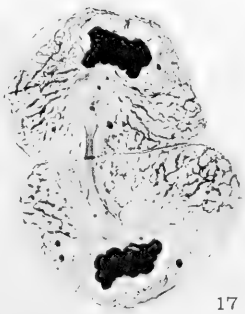

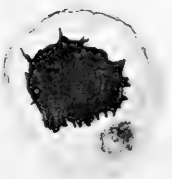

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Fig. 4. Spermatocyte. Transection of the nucleus when growth is nearly completed. Most of the chromosome-loops are cut twice.

Fig. 5. Spermatocytes. Growth nearly completed. Three cells are cut nearly "longitudinally"; one cell shows the idiozome but not the nucleus. The chromosomes are in the form of loops with their free ends toward the idiozome. This is about the same stage as Fig. 4.

Fig. 6. Splitting of the chromosome threads in two adjacent cells. Chromosomes have lost their orientation in relation to the idiozome. Splitting in some of the threads is not continuous.

Fig. 7. Three Spermatocytes, showing ring-formation; some of the chromosomes are twisted as 8's; while fragments only of others are seen. But one cell is cut in the right plane to show the idiozome. The two centrosomes are yet close together.

Fig. 8. Spermatocyte. Section at the beginning of spindle-formation. The centrosomes within the idiozome have moved slightly apart and faint radiations have appeared. The chromosome rings are situated superficially under the nuclear membrane and are cut irregularly.

Fig. 9. Spermatocyte. Stage slightly older than that of Fig. 8. Radiations are more distinct and penetrate the idiozome. Centrosomes more distinct. Chromosomes near the nuclear membrane.

Fig. 10. Stage older than Fig. 9. Two cells are shown with young spindles forming. The outline of the idiozome is still preserved. Note the angle of the spindle axis. The nucleus of one of the cells is cut superficially, and the chromosome forms are well shown. In the other cell, the chromosomes are on the side of the nucleus toward the idiozome.

Fig. 11. Spermatocyte. Later stage; spindle well formed, with but two chromosomes shown at the level.

Fig. 12. Spermatocyte. Deeper section of the same cell shown in Fig. 11. Ring chromosomes are well shown. Note their shape and bending. Two Y-shaped chromosomes are shown.

Fig. 13. Spermatocyte. Spindle at a later stage. The chromosomes are being arranged on the spindle. The rings are bent in a typical manner.

Fig. 14. Spermatocyte. "Equatorial plate" stage, showing a typical spindle. The chromosomes are breaking apart in the equatorial plate. Fused ends of some of the chromatin loops are seen projecting.

Fig. 15. Spermatocyte. An oblique polar view of the daughter-chromosomes as they pass to the pole, showing the second splitting.

Fig. 16. Spermatocyte. Late anaphase. The chromosomes approaching the poles.

Fig. 17. Spermatocyte. Telophase. The chromosomes have become closely massed; a vacuole caps the mass. Mid-body and remains of the spindle are shown.

Fig. 18. Spermatocyte. At the beginning of the growth period, with the nucleus in a "contracted" condition. The chromatin is in a dense mass, still connected with the nuclear membrane by strands. Detail of structure in the nucleus cannot be made out.

PLATES III AND IV.

The figures of these two plates must be reduced ⅕ more (i.e., ⅕ off any diameter) to permit of correctly comparing size with figures on the first two plates.

Division of Spermatocyte II.

The following figures may be compared with the corresponding stages of the Spermatocyte I:

Fig. 19. Secondary Spermatocyte. Two daughter-cells of the first division at the "dispirem" stage. The chromosomes have expanded somewhat; the apices of the V's are still toward the pole of the cells.

Fig. 20. Secondary Spermatocyte. Later stage. Slightly oblique view showing five of the chromosomes, which are seen to be double. They still maintain their polar arrangement.

Fig. 21. Secondary Spermatocyte. Polar view of a similar stage. Eight chromosomes more or less complete are shown. The polar arrangement is still maintained.

Fig. 22. Secondary Spermatocyte. Transection (equatorial section) of the nucleus at a deeper level. The cut chromosome threads are shown; and their arrangement in fours is indicated. The apex of one group of four threads is seen.

Fig. 23. Secondary Spermatocyte. Later stage. Three cells are shown; in one the section is near the middle and the chromosomes, now shortened to X's, are seen to lie superficially. In the other two cells, the section cuts the nucleus nearer the surface.

Fig. 24. Secondary Spermatocyte. Stage similar to that shown in Fig. 23. Crosses and X's are shown.

Fig. 25. Secondary Spermatocyte. Longisection at the beginning of spindle formation. The chromosomes are in the form of crosses and are next the nuclear membrane.

Fig. 26. Secondary Spermatocyte. A later(?) stage. The nucleus is cut superficially. One centrosome only can be seen next the cell membrane.

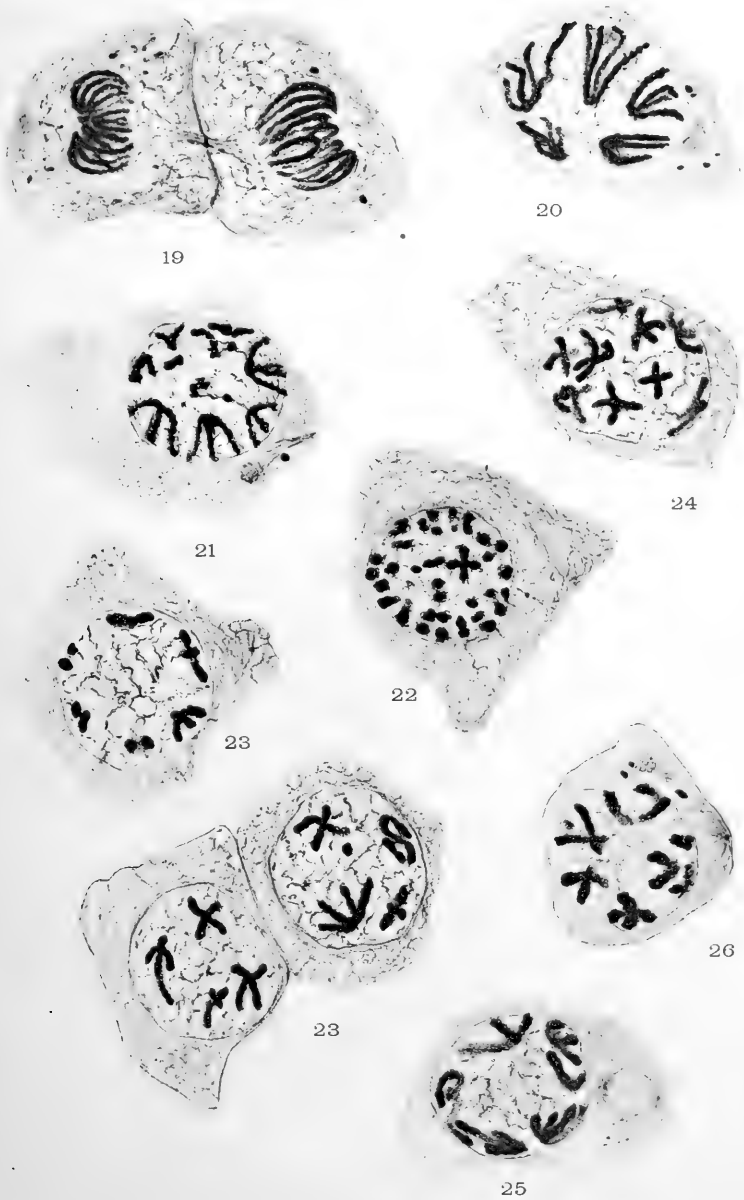
Fig. 27. Secondary Spermatocyte. Spindle formation. The one centrosome is located near the cell-membrane, the other at a distance, near the nucleus, and a spindle is not yet formed between them.

Fig. 28. Secondary Spermatocyte. Later stage; spindle almost completed. Nuclear membrane has been dissolved.

Fig. 29. Group of five secondary spermatocytes. The equatorial plate stage just being established. The crosses in most cases have been dissolved, and the daughter-V's have become applied to each other. Two of the cells are cut somewhat obliquely so that only one pole of the spindle is shown.

Fig. 30. Secondary Spermatocyte. Typical equatorial plate stage. The spindle is quite spherical; mantle fibers are shown.

Fig. 31. Secondary Spermatocyte. Anaphase. Daughter-V's passing to the poles of the spindle.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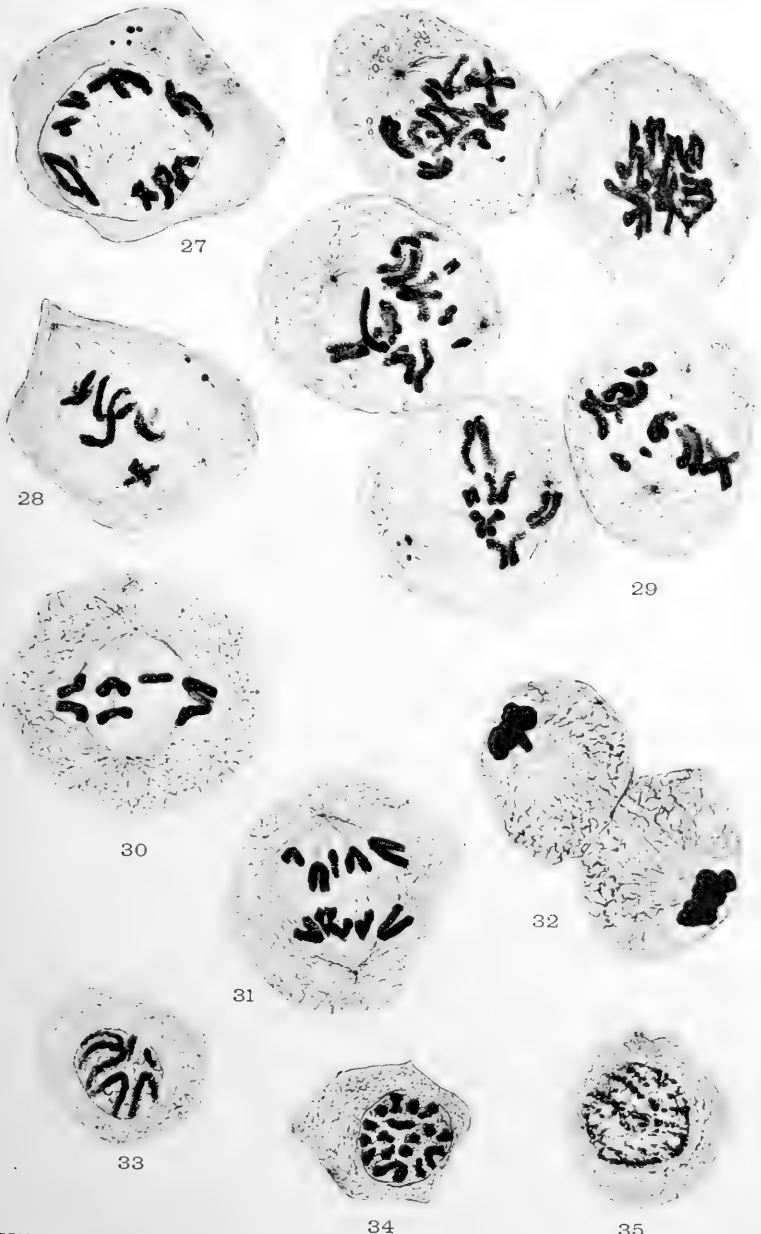
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FIG. 32. Secondary Spermatocyte. Telophase. Chromosomes closely massed at the poles. Division of the cell-body completed. Mid-body and spindle-relic seen.

FIG. 33. Spermatid. Oblique polar view of a daughter-nucleus of the division of Spermatocyte II, at the "dispirem" stage, when the chromosomes again expand.

FIG. 34. Spermatid. Transection of the nucleus at the stage of Fig. 33, showing the legs of the V's cut across.

FIG. 35. Spermatid, fully formed. The chromatin is irregularly distributed in small masses. The two centrosomes at the edge of the cell-body, near the "Sphere."

ON THE ORIGIN OF THE PULMONARY ARTERIES IN MAMMALS.

BY

JOHN LEWIS BREMER, M. D.

From the Embryological Laboratory of Harvard Medical School.

WITH 9 TEXT FIGURES.

The material used in preparing this paper is from the collection of the Laboratory of Embryology at the Harvard Medical School; the original numbers of the series and sections have been preserved. The drawings are from reconstructions, and represent, as it were, casts of the lumina of the arteries without reference to the thickness of their walls. They are all of the same magnification ($\times$ 80 diameters); the arteries are seen from behind, and the pulmonary arches can be followed until they unite to form the truncus pulmonalis, or until, as in Fig. 1, they enter the heart itself.

In 1857, H. Rathke published his monograph, "*Die Aortenwürzeln und die von ihnen ausgehenden Arterien der Saurier,*" in which appear the diagrams of the aortic arches now made more familiar by their reproduction by Kölliker, Hertwig, Quain, and many others, with or without slight modifications. In these diagrams the right and left pulmonary arteries are represented as arising, in lizards and birds, from their respective fifth, or pulmonary, arches, while in snakes and in mammals one fifth arch alone gives rise to both pulmonary arteries, the other arch becoming obliterated; in snakes the right pulmonary arch remains, in mammals the left. Since this monograph there has been, so far as I know, no special investigation into the origin of the pulmonary arteries.

The earliest buds of the pulmonary arteries, in the rabbit, appear in embryos of about 4.0 mm., one bud from each of the pulmonary arches, on the mesial aspect of each. The growth of these buds is at first backward, then downward and inward, giving a small twist, Figs. 1, 2, 3, x, near the proximal end of the pulmonary artery, which seems peculiar to the rabbit. From this twist, the course is straight downward, on each side of the trachea and slightly anterior to it, to the lungs, where the usual branches are given off. During this downward course no branches

are seen. As the arteries increase in length their proximal ends, where they arise from the aortic arches, seem to approach each other actually, as can be seen by comparing Figs. 1, 2 and 3. The mechanism of this change is probably as follows: the truncus pulmonalis is at first short, soon dividing into its two branches, the right and left fifth aortic arches; as it becomes twisted around the aorta, following the turn of the heart, the truncus pulmonalis pulls on the two fifth arches, which are thus crowded together, forming a double tube, and at the same time the two pulmonary arteries, arising from the mesial aspect of the two arches, are

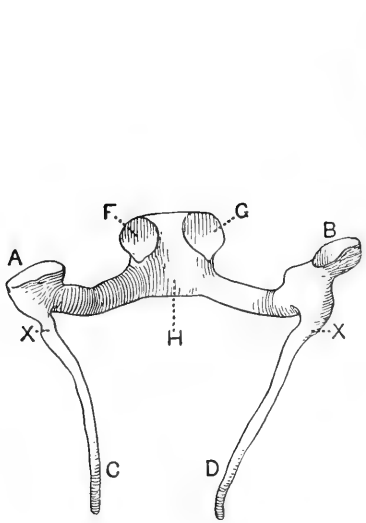

Fig. 1.

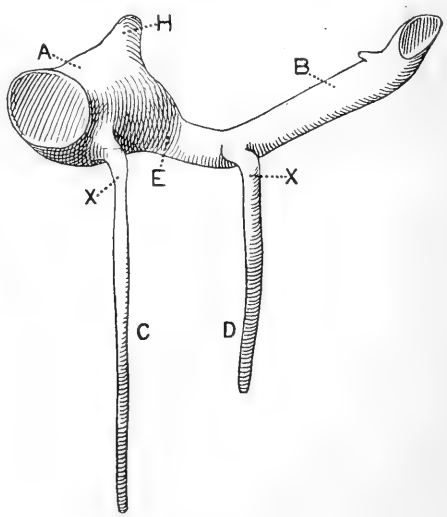

Fig. 2.

Fig. 1. Rabbit of 5.0 mm. Frontal series No. 148, sections 250-261. × 80 diameters. A, B, left and right pulmonary arches, opening directly into heart, H. C, D, pulmonary arteries. F, G, fourth aortic arches, opening into heart.

Fig. 2. Rabbit of 8.0 mm. Frontal series No. 154, sections 291-311. × 80 diameters. E, junction of A and B. H, valve of heart.

brought nearer together. By fusion of the two parallel arches the truncus pulmonalis is increased in length, and its two branches shortened; this fusion may extend until the origins of the pulmonary arteries are very near the bifurcation, or until the left artery springs actually from the bifurcation.

The diameter of the pulmonary arteries remains small in comparison to their increasing length, as one might expect from the slight necessity

of blood in the unused lungs. The left pulmonary arch grows rapidly in diameter as well as in length, while the right becomes entirely obliterated beyond the point where the pulmonary artery arises, leaving finally no trace of its existence; from this point to the junction with the left arch to form the truncus pulmonalis, the right arch remains of the same calibre as the pulmonary artery. The small twist marking the origin of the pulmonary artery gradually straightens out, and the whole right side, *i. e.* the anterior portion of the fifth arch and the

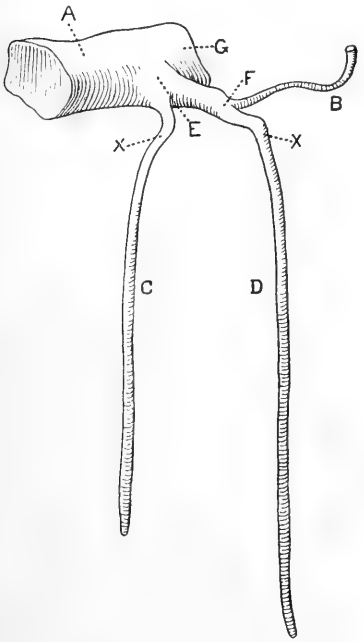

Fig. 3.

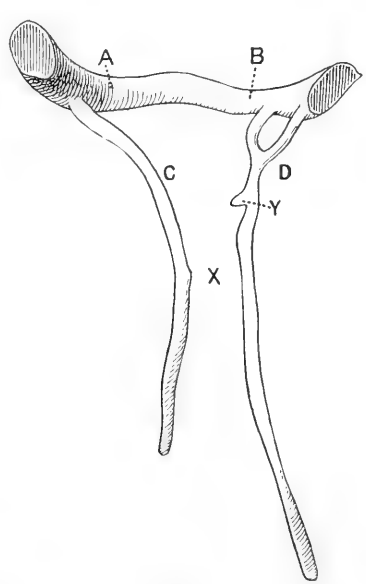

Fig. 4.

Fig. 3. Rabbit of 10.0 mm. Frontal series No. 157, sections 347-367. × 80 diameters.

Fig. 4. Pig of 7.8 mm. Frontal series No. 430, sections 270-297. × 80 diameters.

pulmonary artery, being now unattached to the right dorsal aorta, is drawn to the left by the larger left aortic arch, which is constantly tending to become straight. As a result of these changes, the left pulmonary arch seems to give rise, at about its mid-point, to two arteries, with their origins close together (or there may be a very short common stem); the right one, the longer of the two, arising anteriorly, and taking its course

at first almost horizontally across to the right side of the trachea, then bending down toward the right lung, the left pursuing a straight course to the left lung. The portion of the left fifth arch posterior to the pulmonary arteries becomes later the Ductus Botalli, and is closed at birth.

It will be seen from this description that, in the actual origin of the pulmonary arteries, the rabbit is identical with birds and reptiles, as drawn by Rathke and verified by many other writers. In the rabbit, as well as in birds and reptiles, one pulmonary artery arises from each pulmonary arch, but in birds and reptiles the growth of these arches is equal until birth, so that the picture is symmetrical, a fifth arch, a pulmonary artery, and a Ductus Botalli on each side; while in the rabbit the left pulmonary arch alone remains until birth, and the picture is distorted. It was this distortion, this early disappearance of that portion of the right pulmonary arch posterior to the pulmonary artery, which made possible the diagram of Rathke, and his statement that "in mammals the left fifth aortic arch at a very early period of embryonic life sends out from about its mid-point a small branch which is intended for both lungs, and posterior to its place of origin divides into two twigs."

Rathke examined, of mammals, the pig, sheep, and hare, with special reference to the pulmonary arteries.[1] In the rabbit, cat, and in the few human embryos within my reach, I have found the pulmonary arteries to arise as I have stated, that is, in the beginning, symmetrically, one from each pulmonary arch. In Rathke's original diagrams the arteries of lizards and of birds arise symmetrically, as do they also in the frog, as described by Gaupp.[2] Of snakes, according to Stannius and others, while most species have only the right lung, and therefore only the right pulmonary artery, in adult life, some species have the left lung and left artery alone, and others even both lungs and both arteries, more or less fully developed. In two cases, recently cited by F. Hochstetter,[3] of Tropidonotus tessellatus (a species with only the right lung normally developed), a slender artery was found, which, although finally ramifying in the œsophageal wall, resembled in origin and course a left pulmonary artery. From these facts it seems probable that in the younger snake embryos, of all species, both pulmonary arteries will be found present. If this is the case, the proof will be strong that in all

[1] Müller's Archiv, 1843, p. 276.

[2] Anatomie des Frosches, diagram, p. 285.

[3] Morphologisches Jahrbuch, 1901, p. 419.

vertebrates with lungs the pulmonary arteries originate one from each pulmonary arch, and that Rathke's diagrams, though describing perfectly the adult and late embryonic conditions, are, as regards this origin, incorrect.

In the pig, one of the animals examined by Rathke, although the symmetrical origin is preserved, one pulmonary artery arising from each pulmonary arch, and although the ultimate appearance, that of both

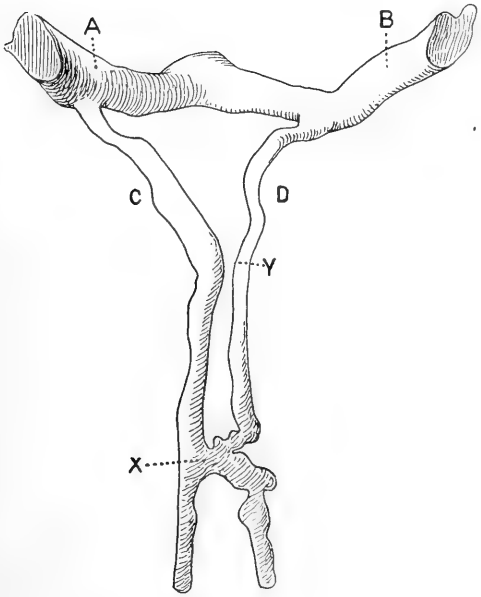

Fig. 5.

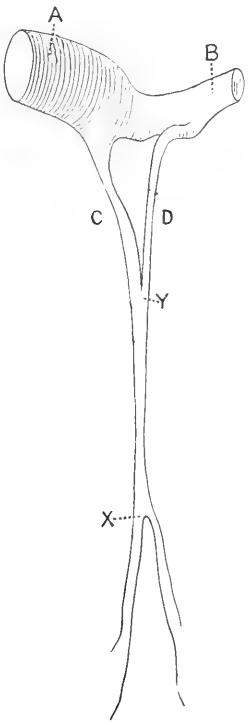

Fig. 6.

Fig. 5. Pig of 9.0 mm. Frontal series No. 54, sections 462-502. × 80 diameters.

Fig. 6. Pig of 11.0 mm. Sagittal series No. 8, sections 96-113. × 80 diameters.

pulmonary arteries arising from the mid-point of the left pulmonary arch, is the same as in the other mammals I have examined, the intermediate steps are different, as is shown in Figs. 4 to 9. Instead of remaining comparatively parallel, as in the rabbit, the pulmonary arteries, after attaining considerable length (pig of 7.8 mm.), bend toward

each other, and instead of remaining without branches (except those developed later in the lungs) send out buds, each toward the other artery, Fig. 4, x, y. This bending toward the median line of these two

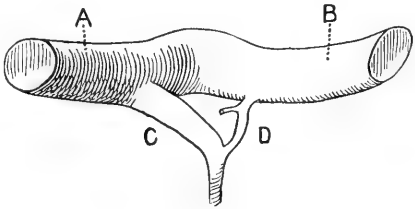

FIG. 7. Pig of 12.0 mm. Transverse series No. 5, sections 366-404. × 80 diameters.

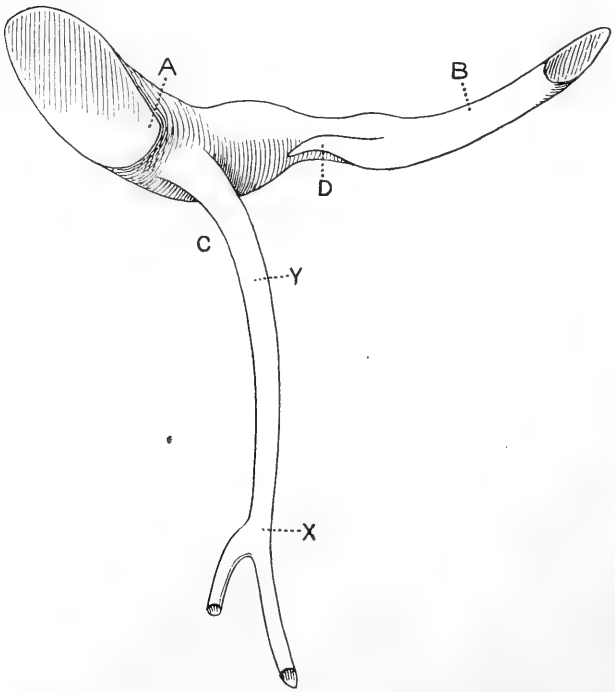

FIG. 8. Pig of 12.0 mm. Frontal series No. 6, sections 429-464. × 80 diameters.

pulmonary arteries is perhaps caused by the great growth of the auricles of the heart in the pig. Both processes continue until in a pig of 9 mm. there is at least one connection between the right and left pul-

monary arteries, often two, as is suggested in Fig. 5, x, y, while in a pig of 11.0 mm. the two arteries, along a considerable part of their length, have merged into one channel, Fig. 6. Meanwhile the upper or proximal part of the right pulmonary artery, which often shows signs of

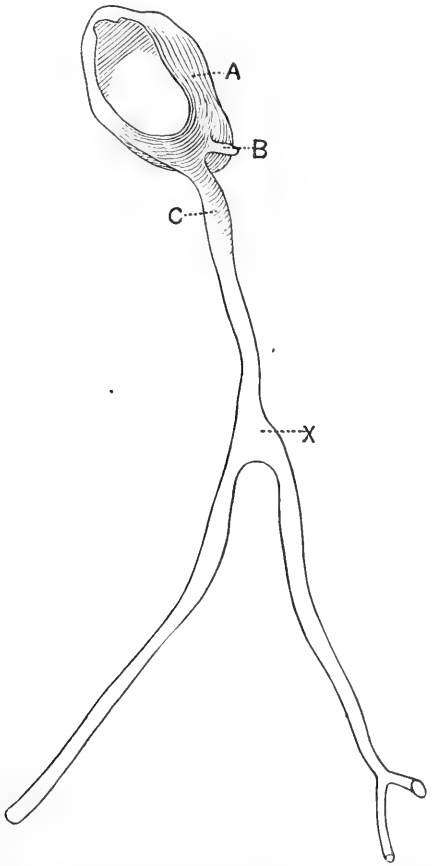

Fig. 9. Pig of 20.0 mm. Frontal series No. 61, sections 270-279. × 80 diameters.

irregularity, such as a double origin, Figs. 4 and 7, D, ceases to increase in size, then grows smaller, and soon becomes obliterated, so that all the blood to both lungs flows through the left pulmonary artery. This gradual change is shown at D, Figs. 5, 6 and 7, and in Fig. 8, where only the remains of the right artery are seen. For a little while after the obliteration of the lumen, a cord of connective tissue marks the

former course of the right pulmonary artery, but soon even this disappears.

Along with this change, another, common to all mammals, has taken place, namely, the obliteration of the right pulmonary arch; but this is not the cause of the obliteration of the right pulmonary artery, since the lumen of the latter is the first to close, Fig. 8. Still another change is seen, as in the rabbit, in the lengthening of the truncus pulmonalis at the expense of the two pulmonary arches, and the consequent apparent movement of the left pulmonary artery toward the right pulmonary arch. In the pig, considerable variation seems to occur in regard to the stage of growth at which this last mentioned change takes place, as may be seen by comparing Figs. 6 and 7, where the distance between the points of origin of the pulmonary arteries is about the same in two pigs of 11.0 and 12.0 mm., respectively, and Fig. 8, where the distance is much greater, although the length of the embryo is again 12.0 mm. It will be seen that of the two 12.0 mm. pigs, one still has, and one has already lost, the connection of the right pulmonary artery.

Whether all ungulates, or only pigs, have this odd method of arriving at the adult relations of the pulmonary arteries, I do not as yet know; certainly there is nothing like it in the rabbit, the cat, the dog, or in the human embryos within my reach.

THE DEVELOPMENT OF THE ARM IN MAN.

BY

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With 2 Plates and 14 Text-Figures.

The wandering of the trapezius and the latissimus dorsi and also of muscles in the abdominal wall was noted by Dr. Mall[1] several years ago. At his suggestion I undertook, in the spring of 1897, a more careful study of these and other changes in the development of the arm region in man. Similar studies were undertaken later by Dr. Bardeen on the leg and body wall. We have embodied many of the important points obtained from our studies in a joint article[2] which appeared in the first number of this journal, and of which this present article may be considered a continuation.

In the present paper I purpose to consider the origin of the tissue which fills the arm bud, the entrance of nerves into this tissue and its differentiation into skeleton, ligaments, muscle and tendon, and finally the growth and wandering of these structures until practically the adult conditions are present.

I wish here to express my most sincere thanks to Dr. Mall for his constant interest and many suggestions, and also for the use of the valuable embryological material in this laboratory.

The embryos studied, with the exception of the one belonging to Dr. Buxton, of Cornell University, are in the collection belonging to Dr. Mall. Most of those considered in this paper are tabulated on page 2, Vol. I, of this journal.

From the serial sections of embryos CLXIII, CIX, XLIII and XXII I have made reconstructions of the arm region after the Born method. The arm region in Plates III to IX in the paper by Bardeen and Lewis

[1] Mall, Development of the ventral abdominal walls in man, Jour. of Morph., Vol. XIV, 1898.

[2] Bardeen and Lewis, Development of the limbs and body wall in man, Am. Jour. of Anat., Vol. I, p. 1.

are drawn from these models, and are to be consulted in connection with the descriptions given in this article. Dr. Mall's embryos are stained in alum carmine or alum cochineal.

PART I.

Relation of the Myotomes to the Arm Bud.

LITERATURE.

Considerable study and perhaps even more theorizing has been done on the relation of the myotomes to the musculature of the limbs. The present state of our knowledge upon this subject is far from satisfactory, especially in the higher vertebrates. The great difficulty or impossibility in many cases of distinguishing between the cells at the ventral edge of the myotomes and those in the neighboring portion of the limb bud renders the problem very difficult. Experimental work, such as has been done by Byrnes,[3] may lead to a clear understanding of the relations in the lower vertebrates. The majority of workers appear to have been able to trace myotomic processes into the limbs. Mollier[4] has shown in Selachians that myotome buds enter into the fin anlage or pectoral plate. From these buds are developed the muscles. From the mesoderm between the buds are developed the fin rays. Braus[5] also shows myotome buds going into the pelvic fins of Selachians. Dohrn[6] finds two buds from each myotome, an anterior and a posterior, entering the fin anlage, these he believes form the fin muscles. Balfour[7] holds that the limb muscles in Elasmobranchii come from muscle plate buds. Harrison[8] has shown that in teleosts the pectoral fins are derived wholly from the somatopleure and that the myotomes take no part in the

[3] Byrnes, Experimental studies on the development of the limb muscles in Amphibia, Jour. of Morph., Vol. XIV, 1898.

[4] Mollier, Zur Entwickelung der Selachierextremitäten, Anat. Anz., Vol. VII, 1892, p. 351. Die paarigen Extremitäten der Wirbelthiere, Anat. Hefte, Bd. III, 1893.

[5] Braus, Beiträge zur Entwickelung der Muskulatur und des peripheren Nervensystem der Selachier, Morph. Jahr., Bd. XXVII, 1899, p. 501.

[6] Dohrn, Studien zur Urgeschichte des Wirbelthier Korpers, VI, etc., Mittheil. aus der Zool. Station zu Neapel, Bd. V, 1884.

[7] Balfour, Comp. Emb., 2nd. Ed., 1885.

[8] Harrison, Die Entwickelung d. unpaaren und paaren Flossen der Teleostier, Archiv f. Mikr. Anat., Bd. XLVI, Heft 3, 1895.

formation of these fins. Boyer[9] believes elements from the peripheral layer of certain myotomes are contributed to the pectoral plate which comes from the somatopleure. Corning[10] believed in 1894 that the pectoral fins in teleosts received muscle-plate buds, but he has since come to the conclusion[11] that these fins in teleosts do not receive such buds, and agrees with Harrison that the myotomes take no part in the formation of the pectoral fins. Kaestner[12] was not able to show in Anura that the myotomes take any part in the formation of the limbs, though he believes they do at a very early period. Field[13] believes that the elements which form the muscle of the extremities in Amblystoma are separated at a very early age from the ventral part of the myotome. Byrnes[14] work, both her embryological and experimental studies, shows that "the myotome processes, as such, take no part in the formation of the limbs . . . The limbs are of somatopleuric origin, *i. e.*, the muscle, cartilage, and connective tissue." Goette[15] believes that the limb muscles in Bombinator develop from the outer layer of the muscle plate. Van Bemmelen[16] believes that in the lizard the limb muscles are derived from the myotome buds. Mollier[17] finds cells from myotome buds go into the arm anlage in Lacerta muralis. According to Patterson,[18] the limbs of the chick are derived wholly from the somatopleure. He does not find muscle buds or homologous structures entering into the limbs. In a recent paper by Maj,[19] the conclusion is reached that the myotomes

[9] Boyer, The mesoderm in Teleosts, etc., Bull. Museum Comp. Zool., Harvard Univ., Vol. XXIII, 1892.

[10] Corning, Ueber die Ventralen Urwirbelknospen in der Brustflosse der Teleostier, Morph. Jahr., Bd. XXII, Heft 1, 1894.

[11] Corning, Ueber die Entwickelung der Zungen Musculatur bei Reptilien, Anat. Anz. (Gesellschaft) 1895.

[12] Kaestner, Extremitäten- und Bauchmusculatur bei Anuren, Archiv f. Anat. und Phys. (Anat. Abtheil.), Hefte 5 und 6, 1893.

[13] Field, Die Vornieren Kapsel, ventrale Musculatur und Extremitätenanlagen bei den Amphibien, Anat. Anz., Bd. IX, No. 23, 1894.

[14] Byrnes, Op. cit.

[15] Goette, Die Entwickelungsgeschichte der Unke., 1875.

[16] Van Bemmelen, Ueber die Herkunft der Zungen- und Extremitätenmusculatur bei Eidechsen, Anat. Anz., Bd. IV, 1889.

[17] Mollier, Die paarigen Extremitäten der Wirbelthiere. Anat. Hefte, Bd. III, Heft VIII; Bd. V, Heft XVI.

[18] Patterson, On the fate of the muscle plate and the development of the spinal nerves in birds and mammals. Quart. Jour. Micr. Sci., Vol. XXVIII, 1887.

[19] Maj, Contributo allo studio dello sviluppo della musculatura negli arti. Osservazioni sur pollo (Gallus domesticus), Dal Bollettino della Soc. Med.-Chir. di Pavia, 1901.

do enter the limbs in the chick. He pictures the ventral end of the myotome entering the limb in company with the nerve and splitting into dorsal and ventral lamellæ. Fischel [20] believes that in birds and mammals myotome cells mix in the limb bud and give rise to the muscles. There is a diffuse entrance of cells from the myotomes but not of myotome buds. In a section of a human embryo of the fourth week he pictures these myotome cells as forming a peripheral layer around the arm bud and even extending into the somatopleure. The rest of the bud comes from the somatopleure. Kollmann [21] pictures in a very diagrammatic manner the downgrowth of the outer lamella of the muscle plate into the arm bud where it lies between the ectoderm and the mesenchymal core.

In the lower vertebrates it would appear therefore that the limb muscles may arise either from distinct buds of the myotome or they may arise independently of the myotomes from the somatopleure. In the higher vertebrates no distinct myotome buds have been traced into the limbs. Myotome cells are supposed by most observers to enter the limbs and take part in the formation of the muscles.

The question as to whether in man the muscles of the arm are derived from cells of the myotomes which have migrated into the arm bud at a very early period, I have not been able to determine satisfactorily. Neither am I convinced by the work of Fischel or Kollmann that the myotomes take such a part in the formation of the arm. Their pictures are quite unlike any of the conditions found in the human embryos which I have studied.

EARLY STAGES OF THE ARM BUD.

In *Embryo CLXIV*, 3.5 mm. in length, there are thirteen myotomes. No signs of an arm bud are present. The myotomes are sharply limited and do not give off any cells into the region where the arm bud is soon to sprout. Cells appear to be migrating from the myotome towards the chorda. The somatopleure, however, shows a proliferation of the cells lining the cœlom. This is very close to the place where the arm bud is soon to appear and lateral to the Wolffian duct and tubules. (See Fig. 1.)

[20] Fischel, Zur Entwickelung der ventralen Rump- und Extremitätenmusculatur der Vogel und Saugethiere, Morph. Jahr., Bd. XXIII, 1895.

[21] Kollmann, Die Rumpfsegmente menschl. Embryonen von 13 bis 35 Urwirbel, Archiv f. Anat. und Phys. (Anat. Abthiel.), 1891.

Embryo XII,[22] 2.1 mm. in length, has fourteen myotomes and is slightly older than CLXIV. The first definite signs of an arm bud are here noticed by a slight swelling ventrolateral to the myotomes in the lower cervical region. Its position is seen in Fig. 2. The origin of the cells which cause this swelling I am not able to determine, though there are suspicious looking processes from the myotomes. No spinal nerves are present.

Embryo LXXVI is 4.5 mm. in length and about three weeks old. Between embryos XII and LXXVI there is quite a gap. There are

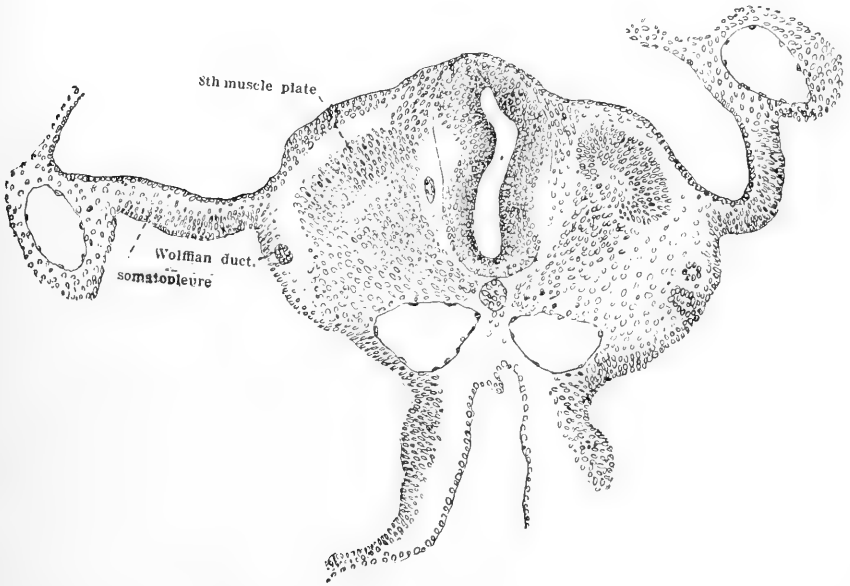

Fig. 1. Cross section through the eighth myotome of embryo CLXIV. × 100 diameters.

35 myotomes. The arm bud is quite large and filled with uniformly and closely packed cells whose nuclei take a deep stain with the alum carmine. A few thin-walled blood-vessels are scattered here and there. The base of the arm bud lies opposite the fifth cervical to the

[22] Dr. Mall considers embryo CLXIV slightly older than embryo XII. The greater length of CLXIV he accounts for by a straightening of the body of the embryo through mechanical injury to the ovum. See Mall, On the development of the human diaphragm, The Johns Hop. Hosp. Bul., Vol. XII, 1901, p. 160.

first thoracic intervertebral disks. The cells of the median lamella of the myotomes have been converted into muscle fibers. The myotomes are fairly well defined and do not show buds, or, so far as I can determine, migration of their cells into the arm bud. The general trend of the growing ventral end of the myotome is not out towards the arm bud, but ventrally towards the cœlom. It will be seen in Fig. 3 that a considerable portion of the root of the arm lies close to the dorsal end of the cœlom and that a proliferation of cells from its lining might easily contribute to the arm tissue. The spinal nerves are not formed though a few anterior root fibers appear to pass directly lateral from the anterior horn. Most of them are lost in the surrounding mesen-

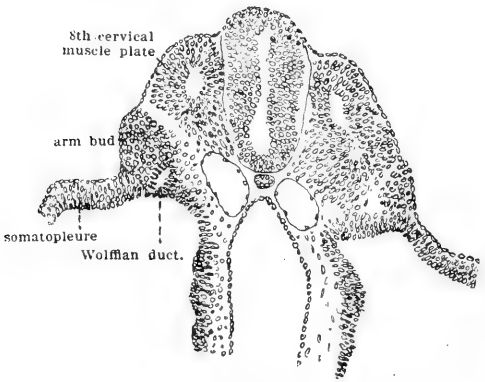

Fig. 2.

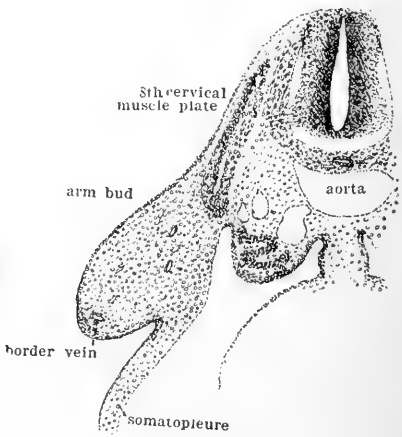

Fig. 3.

Fig. 2. Cross section through the eighth cervical myotome of embryo XII. × 100 diameters.

Fig. 3. Cross section through the eighth cervical myotome of embryo LXXVI. × 50 diameters.

chyma, a few, however, appear to reach the group of muscle fibers on the median surface of the myotomes. This is an exceedingly important stage. The arm bud is filled with a peculiar closely packed mesenchyma which, so far as I am able to judge, is the same sort of tissue from which at a later stage the skeletal and muscular tissues differentiate. This tissue fills the arm before the nerves are developed, and if there are cells from the myotomes present they have migrated there without the nerve supply.

In *Embryo LXXX*, 5 mm. in length, the arm bud has increased con-

siderably in size. The cells which fill it resemble those in LXXVI, but are more closely packed together and stain deeper. There is no differentiation of this mesenchyma. Thin-walled blood-vessels are numerous, the border vein (Randvene of Hochstetter) is present. Numerous mitotic figures indicate that there is a rapid proliferation of the mesenchymal cells. The myotomes are fairly well defined, though in places the ventral end is not always sharp and the possibility of wandering of cells

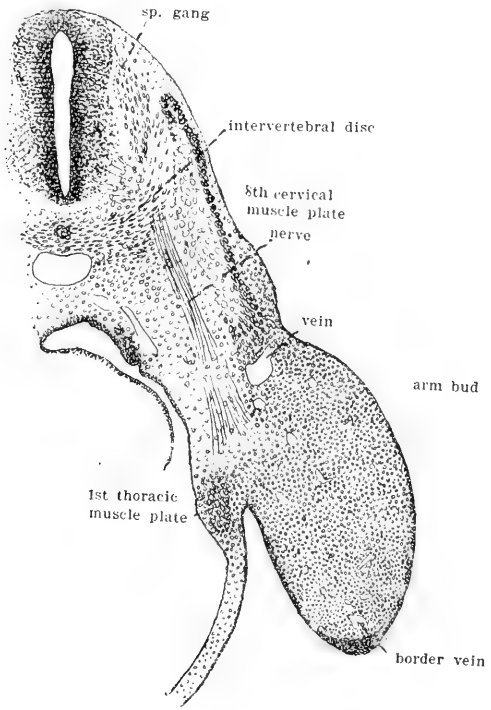

Fig. 4. Cross section through the eighth cervical myotome and nerve of embryo LXXX. × 50 diameters.

from it into the arm bud cannot be denied. The almost constant presence of several blood-vessels at the ventral end of the myotome would interfere somewhat with that process. The spinal nerves have grown out some distance from the cord, they however pass by the median side of the myotomes without sending branches into them. The distal ends of the nerves reach beyond the myotomes. The lower four cervical and first dorsal end at the root of the arm. As will be seen in Fig. 4, this end of the nerve spreads out somewhat and is surrounded here as well

as along its course by loose mesenchyma which is quite different from that in the arm bud, but like that which lies between the myotome and the aorta, and through which the nerve has pushed, probably carrying some of this tissue with it and before it. The first beginnings of the cervical and brachial plexuses are present in the form of anastomoses of the brush-like ends of the first cervical to the third thoracic. It is an interesting fact that at this stage the upper thoracic myotomes extend to a considerable distance ventral of the ventral union of the arm bud

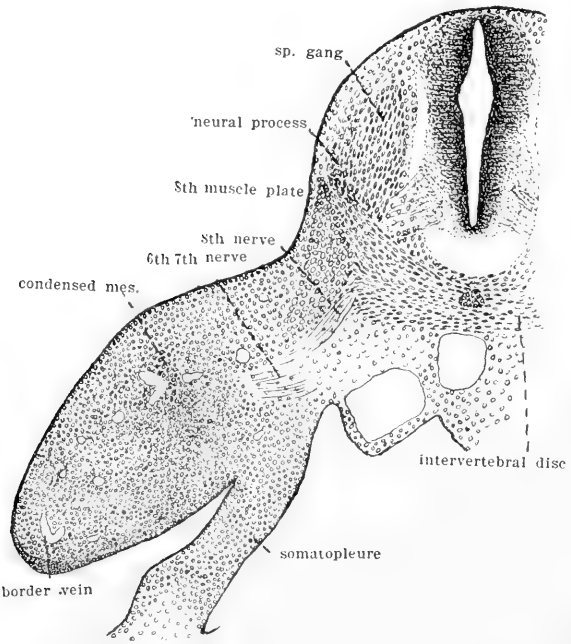

Fig. 5. Cross section through the eighth cervical myotome of embryo II. × 50 diameters.

with the body wall. The tip of one of these thoracic myotomes is seen in Fig. 4. In the cervical region, on the contrary, the myotomes are much shorter and do not extend so far ventrally.

In the *Buxton embryo* about the same conditions exist as in LXXX. Embryo Buxton is 5 mm. in length and about 25 days old. It was stained in hæmatoxylin and eosin, thus bringing out the muscle plates even better than with the alum carmine in which Dr. Mall's embryos were stained.

Embryo II is 7 mm. in length, and about four weeks old. It shows

some advances over LXXX. The arm bud is filled with the closely packed mesenchyma similar to that seen in LXXVI and LXXX, with this important difference however that in the center of the mass the cells are somewhat more closely packed than at the periphery, and represent the first beginnings of differentiation in the arm. This probably represents the humerus. It will be seen from Fig. 5 that the peculiar tissue in the arm bud has spread some distance into the membrana reuniens. Here, as in the previous stages it is impossible to determine whether cells may not go from the myotomes into the arm bud. The nerves, as in LXXX, pass along the median side of the myotome without sending branches into the myotome or, so far as I can determine, taking a portion of the myotome along into the arm bud. They extend farther into the arm than in the preceding stage. The beginning of the cervical and brachial plexus is even more marked, and is formed by anastomoses of the brush-like ends of the first cervical to the second thoracic. The root of the arm lies at the level of sixth cervical to the second thoracic intervertebral discs.

SUMMARY.

The tissue from which the muscles, ligaments, tendons, and cartilages of the arm develop is present at a very early stage in the arm bud, probably by the beginning of the third week. No distinct myotome buds take part in the formation of this tissue. That cells from either or both myotomes and somatopleure enter into this early arm mesenchyma cannot be determined from the material at my disposal. If cells do migrate from the myotomes, they apparently do so independently of the nerves which are not present until the tissue is formed and fills the arm bud. The first beginnings of differentiation of this peculiar tissue which fills the arm bud occur during the fourth week.

PART II.

The Differentiation of the Mesenchyma of the Arm Bud into Muscular and Skeletal Elements, and the Growth of the Nerves.

Embryo CLXIII.

The first indication of a differentiation of the mesenchyma of the arm bud occurs as we have seen in an embryo of about four weeks. In

our next stage, embryo CLXIII, quite marked changes have taken place.

Embryo CLXIII is 9 mm. in length, and about four and one-half weeks old.

In order to gain a clear conception of the form and various relations of the structures in this embryo, I found it necessary to construct a model, and in order to do so it was necessary to draw sharp lines about the various structures when in reality there were no sharp limits. One mass often shading off into another, while the central portion of each was very distinct. Thus, in most places the skeletal core of the arm

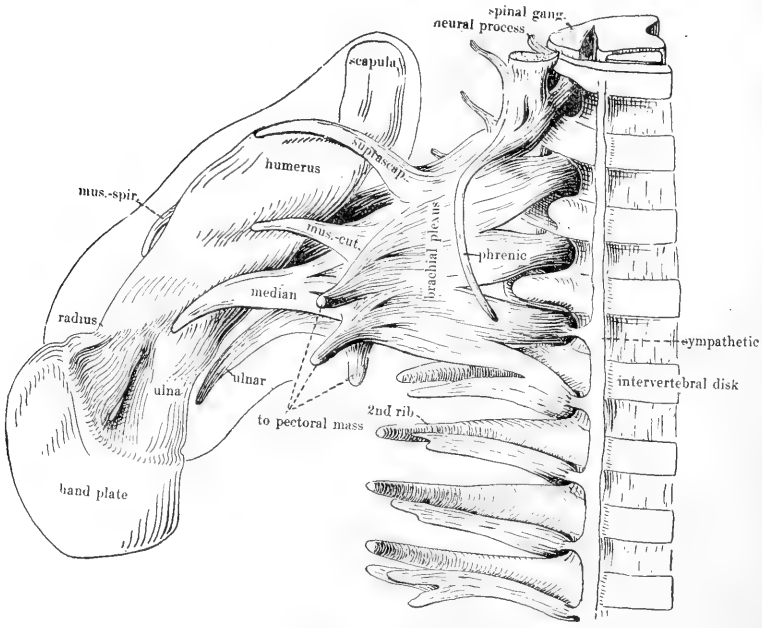

Fig. 6. Skeleton and nerves of the arm region in embryo CLXIII. × 40 diameters.

shades off into the surrounding premuscle tissue, or as in the region of the hand plate into the primitive condensed mesenchyma, filling the distal end of the arm bud. The same was often true of the various premuscle masses. The main portion of these are quite distinct, but they often shade off into each other and into the surrounding mesenchyma. We find in this embryo in the arm region that the premuscle masses most closely associated with the trunk are the farthest advanced, those connecting the arm and trunk next, and the least devel-

oped is the general arm premuscle sheath, especially its distal portion.

I have given the name *premuscle* to various masses of condensed mesenchyma from which at a later period I believe muscle develops by histogenetic changes of the cells.

The Skeletal System.—There is no cartilage at this stage. The skeleton is composed of condensed or closely packed mesenchyma which takes a deeper stain than the surrounding tissue.

The *vertebral column* consists in the arm region of the *intervertebral discs*, and their neural processes which lie in the posterior third of each segment. Between the discs is a loose mesenchyma, the cells of which, as well as those in the disc, have a concentric arrangement about the chorda.

The *ribs* spring from the adjacent portions of the disc and neural process. A line of separation is visible. They take a ventrolateral direction into the body wall. The sixth and seventh cervical intervertebral discs have short rib-like processes.

In the *arm* the exact limits of the skeletal structures cannot be determined as this central core which is easily recognized, shades off into the surrounding mesenchyma, which develops into muscle. The *scapula* is a quadrilateral mass at the level of the fourth and fifth cervical discs. There are no indications of coracoid, acromion or spinous processes. The scapula is continuous with the *humerus*, which is a cylindrical mass occupying the center of the proximal portion of the arm bud. Practically all of it lies at a level anterior to the first rib. At the level of the first rib the humerus is continuous with the *ulna* and *radius*. There is a slight flexion of the forearm. They are short and thick. The ulna is the larger and is more directly a continuation of the humerus. Partially surrounding the ulna and radius is a plexus of blood-vessels which helps to outline them. The continuation of this plexus is seen in Fig. 8. Both ulna and radius are continuous, with the very ill-defined mass of condensed tissue which lies in the center of the distal end of the arm bud. This rather thin plate composed of cells more closely packed together than those of the surrounding tissue, shows no signs of division into the various elements of the hand. I name it the *hand plate*.

The Muscular System.—The *muscle plates* are fused into a continuous column. Indications of segmentation remain. This column lies close and lateral to the neural processes. In the cervical region it ends abruptly at the brachial plexus. In the costal region, however, it extends ventrally into the body wall, between, and partially surrounding the ribs. It ends ventrally beyond the tips of the ribs. The muscle-

plate system is easily distinguished from the surrounding tissues by its fibrillation.

Lateral to the muscle-plate system are ill-defined masses of condensed tissue without fibrillation, but from which muscles differentiate.

Lateral to the anterior six ribs lies the lateral premuscle mass. It occupies most of the space between the costal portion of the muscle-plate system and the integument. It shades off into the surrounding loose mesenchyma everywhere, but at the anterior end, at about the level of the first intercostal space, it splits into four divisions which pass anteriorly.

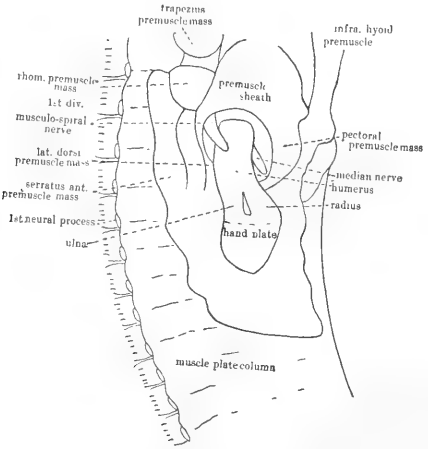

Fig. 7. Outline of the arm region of embryo CLXIII from Plate III. Bardeen and Lewis, Vol. I, No. 1, this Journal. × 15 diameters.

The first or dorsal division lies lateral to the muscle-plate column, and extends to the level of the fifth cervical disc.

The second, third and fourth divisions correspond so closely with the position in which I find certain muscles in the next stage, and as they also have the same nerve supply as the muscles into which I believe they develop, that I have called them in order: The (2) levator scapulæ and serratus anterior, the (3) latissimus dorsi and teres major, and the (4) pectoral premuscle masses.

The second division, the *levator scapulæ* and *serratus anterior* premuscle mass, ventral to the first and opposite the ventral portion of the muscle-plate column is fairly well defined. It extends into the upper cervical region. It lies in a more median plane than the scapula, and at this stage is in no way attached to it.

The third division, the *latissimus dorsi* and *teres major* premuscle mass, passes anteriorly along the dorsal side of the brachial plexus and becomes continuous with the arm premuscle sheath at the proximal

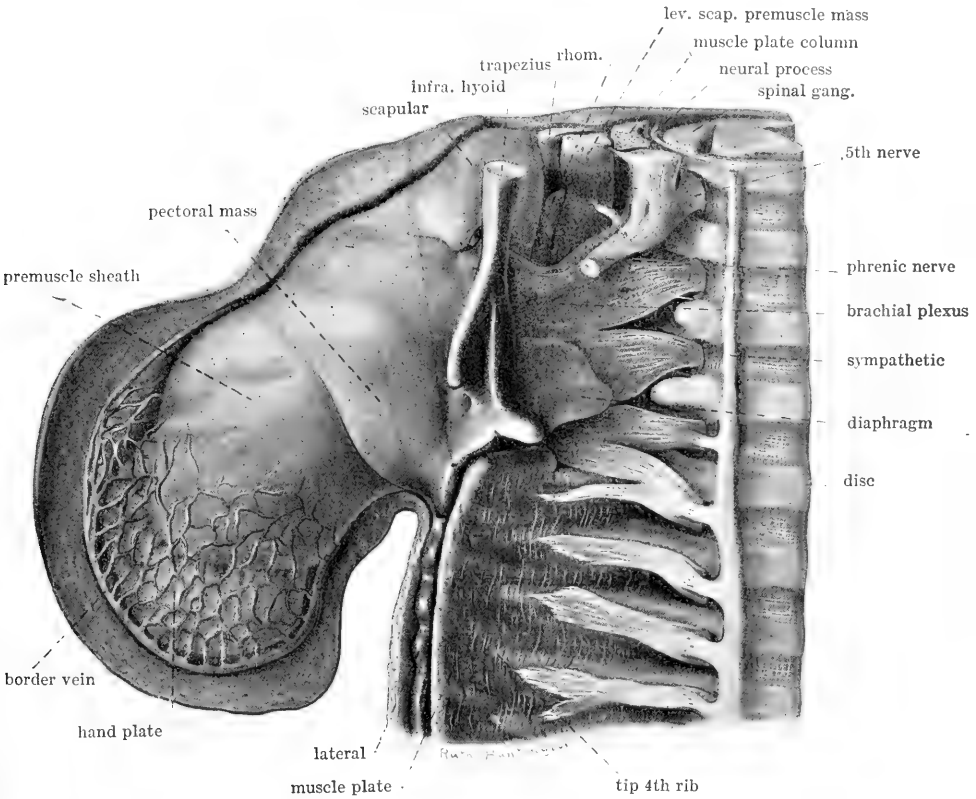

FIG. 8. Ventral view of the arm region of embryo CLXIII. × 40 diameters.

portion of the humerus. The humeral end is thicker and broader, and continuous also with the arm premuscle sheath about the scapula.

The fourth division, the *pectoral premuscle mass*, passes ventral to the brachial plexus and joins the arm premuscle sheath near the proximal end of the humerus. This pectoral premuscle mass is continuous medially with an irregular mass of condensed tissue which extends to the

base of the tongue. The cephalic portion of it is supplied by two nerves, one a branch of the first and second cervical, and the other a branch of the third cervical nerve. These two branches form a loop on the surface of the mass. They correspond to the ramus descendens n. hypoglossus and ramus communicans hypoglossus uniting to form the ansa hypoglossus. Hence I have called this the *infra-hyoid premuscle mass.* I have not been able to determine the fate of the condensed tissue on the median side of the pectoral premuscle mass, and caudal to the infrahyoid mass. The phrenic nerve ends very close to it, and very likely this is *diaphragm premuscle mass.*

The *rhomboid premuscle mass* lies lateral to the second division of the lateral premuscle mass and is an ill-defined plate of condensed tissue. It lies at the level of the fifth cervical vertebra and receives a branch from the fifth cervical nerve, arising in connection with a nerve to the levator scapulæ mass.

The caudal end of the *trapezius premuscle mass* is seen in Fig. 7, lateral to the levator scapulæ mass. The main portion of the trapezius premuscle mass lies opposite the cephalic four cervical vertebræ. It is supplied by the spinal accessory and communicating branches from the first four cervical nerves.

Arm premuscle sheath.—The skeletal core of the arm is surrounded by a mass of tissue which shows no signs as yet of splitting into separate masses. Along the median side of the humerus this sheath is interrupted by the entrance of the brachial plexus and nerves. In places the sheath is separated from the skeletal core by blood-vessels, but in most places no sharp line of separation can be seen. Toward the distal end of the arm the sheath merges into the more primitive mesenchymal tissue which fills most of the distal end of the arm. In Fig. 8 the distal limit of the premuscle sheath is indicated, a portion of the primitive arm mesenchyma having been removed to show the limit of the sheath, the hand plate, the border vein and the venous plexus between the hand plate and the mesenchyma.

The Nerves.—The muscle plate column is supplied by branches of the dorsal rami from all the nerves in this region. They enter the median side of the muscle-plates branch within them, one branch passing through to the subcutaneous tissue.

Branches from the anterior rami of the III, IV, V, VI and VII cervical nerves supply the levator scapulæ and serratus anterior premuscle mass. The rhomboid premuscle mass is supplied by a branch which comes off with the one from the V cervical.

The phrenic nerve arises from the median side of the trunk formed by the IV and V cervical nerves. It does not reach quite to the level of the first rib. See Fig. 6.

The *brachial plexus* is formed from the ventral divisions of the IV, V, VI, VII, VIII cervical and I thoracic nerves. The main portion of the plexus forms a continuous sheet of nerve tissue in which only indications of the three cords can be distinguished. The plexus passes laterally into the arm without any caudal inclination. On reaching the arm it splits into a dorsal and a ventral division. The dorsal division corresponds to the continuation of the posterior cord. It passes around the dorsal side of the humerus, decreasing rapidly in size and ends in the premuscle sheath near the distal end of the humerus. Most of it represents the musculo-spiral nerve. A small branch, which is probably the circumflex, is given off near its beginning. Fibers from all the spinal nerves forming the plexus can be traced into this dorsal division. The ventral division is partially divided into two parts, which probably represent the outer and inner cords. From the outer arises the suprascapular nerve, having fibers from the IV, V, and VI cervical. It passes ventral to the scapulo-humeral junction into the arm premuscle sheath. The rest of this outer cord splits into the musculo-cutaneous and the outer head of the median. The musculo-cutaneous passes into the premuscle sheath on the ventral side of the humerus, and the median into the sheath distal to this, reaching as far as the distal end of the humerus. The inner cord terminates in the ulnar nerve, which runs into the premuscle sheath along the median side of the humerus as far as the beginning of the ulna. Branches going into the pectoral premuscle mass leave the median side of the plexus, one mostly from the outer and the other two from the inner cord. They correspond to the external and internal anterior thoracics. In Fig. 6 the lengths of the various nerves are indicated.

Embryo CIX.

Embryo CIX measures V. B. 10.5 mm. and N. B. 11 mm. in length and is about five weeks old. There is a marked advance over the preceding stage. *Cartilage* has made its appearance both in the vertebræ and in portions of the arm skeleton. There is considerable difference in the character of the cartilage of the vertebræ from that in the arm. The latter seems more advanced and has more the appearance of true hyaline cartilage. It is possible that the cartilage appears first in the arm, though I have not been able to examine intervening stages to

determine this with certainty. Other portions of the arm skeleton are in the precartilage and condensed tissue stages. Both cartilage and precartilage are surrounded in most places by a distinct perichondrium. This takes a very deep stain with the alum carmine. This perichondrium shades off into the condensed tissue of the carpus, which is like that composing the skeletal core in the preceding stage. This again shades into the even less differentiated tissue of the digits, which is at about the same stage of development as the hand plate of the preceding stage, and it in turn shades off into the surrounding mesenchyma.

The muscles in the arm region show very different degrees of development. Those derived from the muscle plate system are in advance of most of the others. The trapezius, levator scapulæ and serratus magnus are about as far advanced as those from the muscle plate system, they show distinct muscle fibers and are for the most part quite sharply limited from the surrounding loose mesenchyma. In position they correspond with their premuscle masses of the preceding stage. The pectoral muscle is next in advance and the latissimus dorsi next. These two muscles grow from the humeral region towards their future attachments on the body wall. It is this portion which lies farthest from the humerus which seems to show the most advance in fibrillation and the sharpest limitation from the surrounding mesenchyma. At the humeral end these muscles gradually shade into a condensed mesenchyma, which fuses with neighboring muscle and skeletal elements. Both muscles correspond in position to their premuscle masses of the preceding stage. As in the preceding stage, embryo CLXIII, the trapezius and serratus premuscle masses were in advance of the pectoral and latissimus; in embryo CIX we find the same relation still continues.

The remaining muscles of the arm apparently develop *in situ* from the premuscle sheath and undergo practically no migrations. They do not appear to be as far advanced as any of the above mentioned muscles. Of these muscles developing from the arm premuscle sheath, the more proximal ones are more developed than the ones more distal. In the scapulo-humeral region most of the muscles show partial fibrillation, while those in the palm of the hand are in about the same condition as the proximal portion of the premuscle sheath in the preceding stage.

The fibrillation, position and nerve supply have made it possible to determine the presence of most of the muscles of the arm.

The Skeletal System.—The *Vertebral Column.* The intervertebral discs are composed of condensed mesenchyma, the cells having a concentric arrangement about the chorda. The vertebral bodies between the discs are each composed of two masses of cartilage, one on either

side of the chorda. They are surrounded by a perichondrium. Along the ventral surface of the vertebral column is a layer of dense mesenchyma, which probably represents both perichondrium and the anterior common ligament. The neural processes, composed of condensed mesenchyma, are clearly defined. They are continuous with the discs and form a wide, shallow groove for the spinal cord. The transverse processes arise by two roots, one from the base of the neural process and the other from the disc. They are of condensed mesenchyma.

The *Ribs.*—The ribs are more sharply defined than in CLXIII. They are of condensed tissue except for a small area near the head, which is of precartilage. They extend farther into the body wall than in the preceding stage.

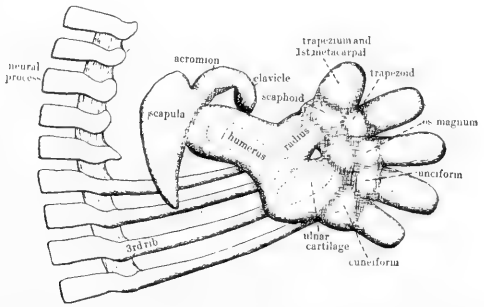

Fig. 9. Skeleton of the arm region of embryo CIX, lateral view. × 12 diameters.

The *Arm Skeleton.*—The *Scapula* is composed of precartilage and has greatly altered in shape. It lies in the region of the lower four cervical and first one or two thoracic vertebræ. From the anterior border, which corresponds to the spine, springs the large curved acromion process. On the median surface at the junction of the humerus with the scapula arises the large hooked coracoid process. Running across the median surface of the scapula to the vertebral border is a slight ridge which separates the supraspinatus from the subscapularis muscles and corresponds to the future anterior border. The condensed tissue is thickened on the medial surface into a perichondrium, while on the lateral surface the precartilage shades off into the surrounding mesenchyma.

The *Clavicle.*—A rather poorly defined mass of condensed tissue continues from the tip of the acromion toward the tip of the first rib, extending for about one-third this distance. This mass represents the clavicle. From it a mass of ill-defined tissue extends to the coracoid process and represents the coraco-clavicular ligament.

The *Humerus* is directly continuous with the scapula and root of the coracoid process. No signs of joint surfaces or cavity are present. Both ends of the shaft are enlarged and the distal end shows both external and internal condyles. The core of the shaft is of hyaline cartilage; this is surrounded by very thick perichondrium, which shades off into the condensed tissue of each end in which is enclosed an area of precartilage. The distal end seems more advanced than the proximal.

The *Radius* and *Ulna* are continuous with the distal end of the humerus, no indications of joint surfaces or cavities being present.

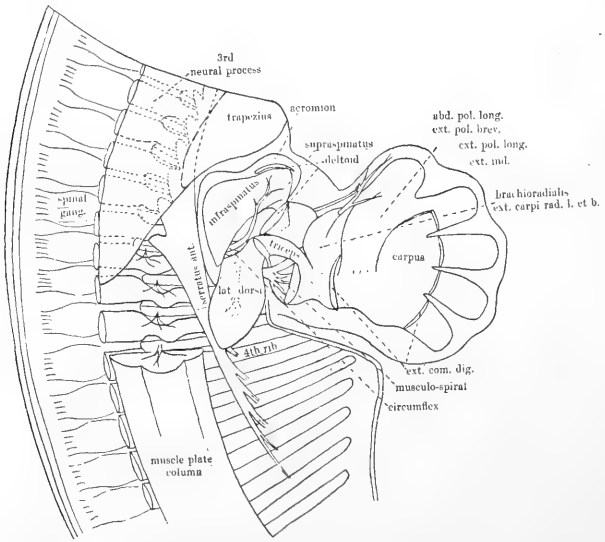

FIG. 10. Outline of the arm region of embryo CIX, lateral view from Plate IV. Bardeen and Lewis, Vol. I, No. 1, this Journal. × 12 Diameters.

There is more flexion at the elbow than in CLXIII. The forearm occupies a position about half way between pronation and supination. The core of each shaft is composed of hyaline cartilage. This is surrounded by a very thick perichondrium, which continues into the condensed tissue at either end of the bone, in which precartilage is enclosed.

The *Hand-plate* is continuous with the distal ends of the radius and ulna. It is composed of condensed mesenchyma. There are several centers of increased condensation which I believe must correspond to the carpal bones, namely, the scaphoid, lunar, pyramidal, trapezium, trapezoid, os magnum and unciform. The scaphoid is in line with the radius and the lunar with the ulna, while the pyramidal is at the ulnar side of

the carpus, and as the metacarpal V continues from it more than the unciform the whole hand has a peculiar bend toward the ulnar side. From the carpus five masses of condensed tissue project. They shade off into the surrounding mesenchyma which fills the distal end of the arm. The condition of these finger masses corresponds to the condition of the hand-plate in CLXIII. There is not the slightest indication of segmentation into metacarpals and phalanges. The radial of the five projections probably consists of both trapezium and metacarpal I, which have not yet shown signs of separate centers of condensation.

The Muscular System.—The *muscle plate system* has become differentiated into several muscles, namely, the deep dorsal muscles, the intercostals, the abdominal muscles and the deep ventral neck muscles.

The *infrahyoid* muscles correspond in position and nerve supply with the infrahyoid premuscle mass of the preceding stage. They extend nearly to the region where the median end of the clavicle will eventually extend.

The *trapezius* muscle has extended posteriorly to the level of the fifth cervical vertebra. Its posterior end lies near to the lateral surface of the body and is connected to the tips of the neural processes as far posteriorly as the second thoracic vertebra by a considerable interval of fascia. As the muscle passes anteriorly it lies deeper and deeper from the surface, being separated from it by the platysma and facial muscles. Its ventral border is free from attachment to the scapula and clavicle. At the level of the second cervical vertebra it is joined by the sternomastoid muscle, which has ascended from the more ventral neck region. The nerve supply is as in the adult.

The rhomboid mass lies in the region of the V and VI cervical vertebræ. It connects with the fascia passing to the dorsal tips of the neural processes but has no scapular attachment. A branch of the fifth cervical nerve supplies it.

The *levator scapulæ* and *serratus anterior* muscles form a continuous fibrillated mass, extending from the first cervical vertebra to the ninth rib. It occupies much the same position that its premuscle mass did in embryo CLXIII except that the posterior end now extends to the ninth rib. Digitations go to all the cervical transverse processes and to each of the anterior nine ribs. The anterior and posterior digitations are very slender and contain but few fibers. The thickest part of the muscle lies in the scapular region. There is no scapular attachment. The ventral edge of the muscle lies at about the same level as the dorsal edge of the scapula but in a more median plane. Branches from the second to the seventh cervical nerves supply the muscle. The first three

penetrate directly into the muscle. The last three form a trunk which runs along the lateral surface of the muscle as far as the fourth rib.

The *pectoralis major* and *minor*[23] are united into a common muscle mass, which is well differentiated from the surrounding tissue. It forms a thick oval mass, which extends from the level of the second rib to the proximal portion of the humerus. The greater part of the muscle thus lies anterior to the first rib. As the mass bends towards the humerus it is attached also to the clavicle. So probably both sterno-costal and clavicular portions are present. The median side of the mass bulges towards the coracoid process and represents the minor. Most of the mass shows distinct fibrillation, but toward the humerus this passes into the condensed tissue which is not sharply outlined from the surrounding structures. The position of the pectoral muscle corresponds to the position of the pectoral premuscle mass in embryo CLXIII. Branches from the median side of the brachial plexus supply the pectoral. Two from the external cord contain fibers from the fifth, sixth and seventh cervical nerves. Two come from the inner cord. Within the muscle complicated anastomoses occur from which fibers spread out in all directions.

The muscles thus far considered were fairly definite, and, as we have seen, come from quite definite premuscle masses. The remaining muscles of the arm are in process of differentiation from the arm premuscle sheath. The exact limits of the individual muscles are almost impossible to determine.

The *deltoid* muscle extends from the acromion and clavicle and fascia over the infraspinatus to the humerus. It is very closely connected with the infraspinatus and only by the difference in the nerve supply can the two be separated. The position of the teres minor is also only indicated by its nerve and not by any line of separation between it and the infraspinatus or deltoid. The origin of part of the deltoid from the acromion and clavicle helps to distinguish some of its fibers, but a short distance from this origin no line of separation can be made between it and the infra- and supraspinatus muscles. Condensed tissue connects it with the triceps and pectoral muscles. The circumflex nerve supplies this muscle and also sends a branch to fibers which are closely associated with the infraspinatus and probably constitute the teres minor muscle.

That portion of the *infraspinatus* which lies on the lateral surface of the scapula is fairly distinct except where the deltoid and teres minor

[23] Lewis, Observations on the pectoralis major muscle in man, Johns Hopkins Hosp. Bul., Vol. XII, 1901.

muscles join it. The portion of the *supraspinatus* on the anterior one-fourth of the median surface of the scapula is distinct, but after it passes the acromion it is inseparably connected with the infraspinatus and deltoid and pectoral muscles. These muscles shade off into the proximal end of the humerus. The main portion of each of these muscles contains muscle fibers. The suprascapular nerve supplies the supra- and infraspinatus muscles.

The *subscapularis* muscle arises from the posterior one-half of the median surface of the scapula and passes beneath the coracoid process to the humerus. The circumflex nerve separates a portion of it from the teres major muscle, but the scapular portions of the two are closely united, as is also the long head of the triceps. A branch from the circumflex and another from the posterior cord of the brachial plexus supply the subscapularis.

The *teres major* and *latissimus dorsi* muscles are closely associated at their humeral end. The latissimus dorsi lies in the lateral thoracic region, extending posteriorly as far as the fourth rib. It has no attachments to the ribs or vertebral column. The two muscles are inserted together into the proximal portion of the humerus. The teres major arises from the axillary border of the scapula near its posterior angle. The common portion of the latissimus and teres passes close to the posterior cord of the brachial plexus, from which a large branch is given off that runs into the latissimus and has a brush-like ending near the posterior limit of the muscle. A smaller branch of the posterior cord is given off to the teres major.

The *triceps* muscle extends along the posterior and lateral surfaces of the humerus, extending from the scapula to the ulna. Indications of the three heads are present. The portion of the muscle lying near the insertion of the latissimus dorsi and the infraspinatus muscles is not sharply defined from them. The musculo-spiral nerve passes through the muscle and gives branches to it.

The *biceps* and *coracobrachialis* muscles lie along the median side of the humerus, extending from the coracoid process to the radius. The two heads of the biceps are quite closely united nearly to their origins, which are but a short distance apart. The portion of the coracoid process from which the long head arises must ultimately become a portion of the head of scapula. The attachment of the coracobrachialis to the humerus is by condensed tissue, as is the distal end of the biceps to the radius. The distal end of the biceps blends with the brachialis and the flexor mass. The musculo-cutaneous pierces this group and gives off branches to it.

The *brachialis* muscle is closely attached to the distal one-half of the humerus over the anterior and median surfaces. It is also closely attached to the overlying biceps muscle and it is impossible to determine just the line between the two or between it and the brachioradialis muscle. It is also impossible to determine the exact line between the muscle and the underlying perichondrium. It is closely associated with the triceps on one side and the deltoid on the other. The main portion of the muscle is fibrillated and is inserted into the ulna by condensed tissue, which is closely associated with the flexor mass of the forearm. The musculo-cutaneous nerve gives off a large branch which has a brush-like ending within the muscle.

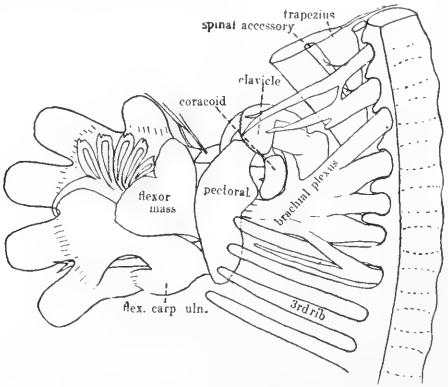

Fig. 11. Outline of the arm region of embryo CIX, median view, from Plate V. Bardeen and Lewis, Vol. I, No. 1, this Journal. × 15 diameters.

The *flexor muscle mass* of the forearm forms a thick layer over the median surface of the ulna, radius, carpus and proximal end of the metacarpus. It is with considerable difficulty that I have separated this mass into two layers. The *superficial layer* is smaller in extent and lies in the proximal region of the forearm. It is connected with the radial portion of the forearm by a condensed tissue mass and distally fuses with the deep layer to become continuous with the condensed tissue of the digits. The median nerve passes through the proximal portion and then comes to lie between the two layers. From its position and relation to the median nerve I believe this to be the layer from which the flexor carpi radialis, flexor sublimis digitorum, pronator teres and palmaris longus muscles differentiate. Branches from the median nerve supply this layer. Both layers arise partly from the inner condyle of the humerus, and are continuous more or less with the muscles of the upper arm. The *deep layer* is closely attached to the perichondrium of

the forearm and hand. It is wider in extent than the superficial and shows indications of separations into muscles. The portion for the flexor carpi ulnaris shows most advance. The extension into the hand probably constitutes the portion from which the interossei and lumbrical muscles and flexor tendons develop. It is continuous with the condensed tissue of the digits. The portion on the forearm forms the flexor profundus digitorum, flexor pollicis longus, flexor carpi ulnaris and pronator quadratus muscles. Both the ulnar and median nerves supply the deep layer.

The *extensor mass* of the forearm is farther advanced than the flexor. It can be differentiated into three groups of muscles which accord well with the adult groups. The *first group,* the largest and most superficial, extends from the lateral condyle to the proximal ends of the digits, where it blends with the condensed mesenchyma. It is a thin layer and spreads out over the ulnar two-thirds of the forearm and is quite closely applied to the perichondrium and condensed mesenchyma of the skeletal structures beneath. A portion of it overlaps the second and third groups. It is the still undifferentiated *extensor communis digitorum, extensor carpi ulnaris,* and *extensor minimi digiti.* It is supplied by branches of the posterior interosseus nerve.

The *second group* occupies the proximal portion of the radial side of the forearm. It arises in connection with the first group from the external condyle and adjoining portion of the humerus. The muscle mass passes distally along the radius and soon divides into two parts between which the radial nerve passes. The radial part fuses with the condensed tissue of the distal end of the radius. It is the *brachioradialis* muscle. The second part passes beneath the third group and fuses with the condensed mesenchyma at the proximal ends of the second and third digits. It is the *extensor carpi radialis longior et brevior muscle.* Branches of the musculospiral nerve supply this second group.

The *third group* arises beneath the first from the ulna and radius. Its fibers pass toward the radial side of the forearm, passing from beneath the first group and over the second group, and finally end in the condensed tissue of the first and second digits. The portion to the second digit is closely fused with the portion of the first group which goes to this digit. This group is quite closely applied to the underlying skeletal condensed tissue. The third group represents the abductor pollicis longus, extensor pollicis brevis, extensor pollicis longus and extensor indicis proprius. Branches of the musculospiral nerve supply this group.

The *supinator* I believe must arise in connection with the third group, judging from its position and the direction of its fibers.

The muscle fibers of the extensor groups do not extend as far distally as do those of the flexor mass.

The Nerves.—The enormous size of the lower cervical nerves attracts the attention at once. In the plates and figures they are given in their true proportion to the other structures. The main portion of the brachial plexus has but a very slight posterior inclination.

A branch from the V cervical supplies the rhomboid muscle mass.

The V, VI, VII and VIII cervical and I thoracic nerves unite to form the brachial plexus. The IV cervical does not connect with the plexus. The main portion of the plexus forms a continuous sheet in which indications of the three cords can be seen. The V and VI unite before joining the others and from this union is given off the suprascapular. It leaves the trunk at right angles and has the appearance of having its proximal end dragged distally toward the arm by the main portion of the plexus. The VIII and I thoracic unite before joining the plexus. The continuous sheet formed by these five nerves soon splits into a lateral (dorsal) and median (ventral) division. The lateral corresponds to the posterior cord and from it arise the circumflex, subscapular and musculospiral nerves. These nerves take the normal course found in adult and supply the same muscles as in adult. Cutaneous branches are also given off. The median sheet of the plexus quickly divides into several bundles. The anterior one corresponds to the distal end of the external cord. From it are given off the musculo-cutaneous, two branches to the pectoral mass, and one head of the median nerve. The posterior division corresponds to the distal end of the inner cord. From it arise branches to the pectoral mass, the inner head of the median, the ulnar and internal cutaneous nerves. The distal end of the median splits into a peculiar fan-like arrangement of its branches. Both median and ulnar give branches to the deep flexor mass and anastomose within the mass.

I have attempted to trace the origin of the fibers in the main nerves of the arm. The results are given in the following table:

	Cervical.	Thoracic.
Suprascapular	V, VI ?	
Subscapularis	V, VI, VII	
Long thoracic	VII, VIII	I
Anterior thoracics	V, VI, VII, VIII	I
Musculo-cutaneous	V, VI, VII ?	
Median	V, VI, VII, VIII	I
Circumflex	V, VI, VII	
Musculospiral	V, VI, VII, VIII	I
Ulnar	VI ?, VII, VIII	I

Embryo XLIII.

Embryo XLIII measures 16 mm. V. B. and 14 mm. N. B. It is about six weeks old. Many changes have taken place during the sixth week. The entire arm has migrated posteriorly, dragging muscles and nerves with it. The brachial plexus has a decided posterior inclination. The skeletal system is much farther advanced and consists for the most part of cartilage; its individual elements are assuming more the adult form. The clavicle now unites the arm and thoracic skeletons.

The muscular tissues have become more clearly differentiated and except in the hand are easily distinguished. Muscles, such as the trapezius, serratus, and pectoral, have spread out into sheets and acquired more their permanent attachments, in the case of the trapezius, latissimus and pectorals by migration or extension of their fibers.

In the hand, however, we find the interossei still in an undifferentiated condition like that of the deep flexor layer in embryo CIX or the serratus and infrahyoids in embryo CLXIII.

The Skeletal System.—The *vertebral column.* The intervertebral discs are of still more compact tissue than in embryo CIX, but they occupy only about one-fourth height of the segment, while in CIX they occupied nearly one-half the anterior-posterior length of the segment. The body of each vertebra contains a large mass of cartilage, which is continuous with the cartilage in the transverse and neural processes. Indications of the hypochordal brace of Froriep are present in connection with the ventral side of the first three discs. The anterior one is the largest, the others decreasing rapidly in size.

The *ribs* are composed of long, slender cartilages, surrounded by a thick perichondrium. This is continuous with the condensed tissue of the tips of the ribs. The tips of the first seven ribs are connected by a narrow strip of condensed tissue which appears to be formed by the turning anteriorly of their tips until they touch the rib above and fuse with it. Thus is formed the *anlage* of one-half the *sternum* on either side some little distance from the median line. There is at present no sign of union of the two halves of the sternal *anlagen.* The first rib is fused with the median end of the clavicle. The ribs show a marked increase in their lateral convexity, as in embryo CIX there was scarcely any. There are no joint cavities between the ribs and vertebræ.

The *scapula* is composed largely of cartilage. It has migrated posteriorly so that less than one-half of it lies above the level of the first rib. The whole scapula is larger than in embryo CIX. There is a thick layer of perichondrium around the cartilage and a considerable

mass of condensed tissue along the vertebral border, and at the posterior angle, the cartilage reaches to the level of the third rib and the condensed tissue nearly to the fifth. The anterior border is somewhat irregular and thickened and gives origin in part to the supraspinatus muscle. The lateral lip of this border probably represents the spine and the median lip the anterior border. Projecting from the lateral side of the head and continuous with the lateral lip of the anterior border is the acromion process. It is large, curved and mostly of condensed tissue and contains a slender core of cartilage continuous with the cartilage

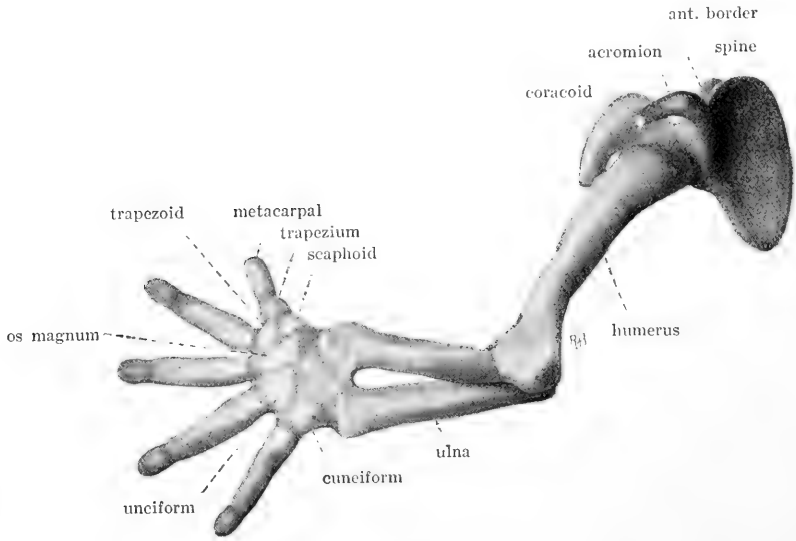

FIG. 12. Cartilaginous skeleton of the arm of embryo XLIII, lateral view. × 20 diameters.

of the body. The coracoid process arises from the median side of the head, is larger than the acromion, and contains a much larger cartilaginous core, which is continuous with the cartilage of the body. The acromio-clavicular ligament is strongly developed.

The *clavicle* consists of a thick mass of condensed tissue, extending from the acromion to the tip of the first rib, where it continues with the half sternal anlage. There is no line of separation at either end. There is a small core of a peculiar precartilaginous tissue.

The *humerus* is larger, longer and more slender in proportion to its

length than in the preceding stage. The two ends are enlarged. The main portion is of cartilage surrounded by a thick perichondrium which is continuous with that of the head of the scapula, forming the beginning of the capsular ligament. There is also a strip of perichondrium between scapula and humerus in which there are no signs of a joint cavity. At the proximal end the perichondrium shows thickenings for the tuberosities, while at the distal end the condyles are for the most part of cartilage continuous with that of the main portion. Considerable masses of condensed tissue, however, help to increase the size of the condyles. A portion of the head of the humerus rests against the base of the coracoid process, indicating that a portion of this is to be incorporated with the head of the scapula.

The *ulna* and *radius* are of cartilage surrounded by a thick perichondrium. This is continuous with that of the distal end of the humerus, forming the beginning of the capsule. The perichondrium of the proximal end of the radius is continuous with that of the adjoining surface of the ulna. The cartilages of the humerus, radius and ulna are separated from each other by condensed tissue in which no signs of cavities are present. The olecranon is quite well developed and consists mostly of cartilage. The coronoid process is mostly of condensed tissue. The great sigmoid fossa is rather shallow. The bicipital tuberosity is of condensed tissue. The distal ends of these bones are enlarged and separated from each other by condensed tissue continuous with the perichondrium of each.

The *carpus* consists of a condensed tissue matrix in which lie imbedded the various cartilages. The distal row is complete, the trapezium, trapezoid, os magnum and unciform. The latter has spread in between the fifth metacarpal and the cuneiform (pyramidal). In the proximal row the cuneiform and scaphoid are of cartilage and the lunar and pisiform of condensed tissue.

The *metacarpus* shows five slender cartilages surrounded by very thick condensed tissue layer or perichondrium. The first metacarpal cartilage is only about one-half the length of the others.

The ulnar four *phalanges* of the first row are present as short slender cartilages deeply imbedded in condensed tissue. In the first digit condensed tissue takes the place of the cartilage. At the tip of each digit is a mass of condensed tissue.

There are no joint cavities between the cartilages of the hand, each one is separated from its neighbor by an area of condensed tissue.

Hagen[24] has reconstructed the cartilaginous skeletal system of a human embryo of about this age. A comparison of the drawings from the reconstructions shows that there is considerable variation in the carpal region. In none of my stages does the metacarpal come in contact with the radius, either before or after the cartilages of the carpus and metacarpus appear, and there is a considerable area of dense mesenchyma between metacarpus and radius. I am inclined to believe what he calls metacarpal I, may be trapezium and his so-called first-phalanx the metacarpal.

The Muscular System.—*Plates I and II, Figs. A and B.* The *trapezius* muscle has both clavicular and acromial attachments. The muscle has extended posteriorly so that the muscle fibers run from the occiput to the level of the fifth rib. They are connected by a considerable interval of fascia with the dorsal ends of the cervical and all the thoracic neural processes.

The *levator scapulæ* and *serratus anterior* muscles are greatly altered in shape. The latter forms a broad, thin sheet between the dorsal border of the scapula and the first nine ribs, being attached by a digitation to each rib. The scapular attachment is into the condensed tissue along its dorsal border.

The *pectoral* mass is now spread out into a large, thin sheet, which has split into the major and minor muscles. The clavicular and sterno-costal portions of the *pectoralis major* are separated by a considerable interval. The clavicular fibers arise from the median one-third of the clavicle and pass to the humerus. They overlap the humeral ends of the sterno-costal fibers which arise from the first six ribs and the sternal anlage.

The *pectoralis minor* is a distinct muscle arising from the second, third and fourth ribs and passing to the coracoid process.

The subclavius muscle is quite well developed and runs from the first rib to the clavicle, having a course nearly at right angles to the latter.

The *latissimus dorsi* has spread out into a broad, thin sheet of muscle fibers, which are connected by fascia with the lower thoracic and lumbar neural processes. Its humeral end is closely united with the teres major.

The *teres major* muscle has about the relations found in the adult. It and the latissimus dorsi are inserted together into the humerus.

The *deltoid* muscle is very much like the adult in its attachments and shape.

[24] Hagen, Die Bildung des Knorpelskeletes beim menschlichen Embryo, Arch. für Anat. u. Phys., 1900.

The *infraspinatus* muscle arises from the anterior portion of the lateral surface of the scapula and can be easily traced to its insertion into the great tuberosity of the humerus. The teres minor cannot be separated from it.

The *supraspinatus* muscle arises from the anterior thickened border of the scapula and passes to the great tuberosity of the humerus.

The *subscapularis* muscle occupies the central portion of the median surface of the scapula. It is separated from the teres major. It passes beneath coracoid process to the lesser tuberosity of the humerus.

The *triceps* muscle is easily traced from its origin by the three heads to its insertion into the olecranon process. The three heads are quite easily distinguished. The long head is smaller in proportion than in the adult.

The *biceps* muscle is more elongated and shows more of a separation of its two heads than in embryo CIX. The long head still arises from the base of the coracoid process. The two heads join about the middle of the humerus and pass to a thickening of condensed tissue on the radius. The short head arises in common with the coracobrachialis muscle from the tip of the coracoid process. This latter muscle is inserted into the middle of the median surface of the humerus. It is closely connected with the biceps for most of its length.

The *brachialis* muscle is spread out more over the distal portion of the humerus and its muscle fibers extend farther toward the insertion into the coronoid process of the ulna than in the preceding stage.

The *flexor mass* of the forearm and hand show a most marked advance over the preceding stage. The various muscles of the superficial layer which arise from the internal condyle are easily recognized. They are more or less fused at their origin and for some little distance from it.

The *palmaris longus* muscle, the most superficial one, is thin and wide, ends in the condensed tissue of the palmar fascia.

The *pronator teres* muscle passes to the middle of the shaft of the radius.

The *flexor carpi radialis* muscle lies mostly on the radial side of the forearm, towards the distal end of which it bends under the deep flexor and ends in a condensed tissue tendon which fuses with the condensed tissue near the proximal end of the second metacarpal. This portion of the muscle is not yet clearly differentiated from the condensed tissue on the palmar surface of the carpus.

The *flexor digitorum sublimis* muscle arises beneath the palmaris longus in connection with it from the internal condyle, and also from the shaft of the ulna, for a little distance distal of the coronoid process.

It is very broad and spreads out over the middle of the forearm and carpus, where it divides into four broad, thin tendons which fuse with the condensed tissue surrounding the distal end of the four ulnar metacarpals and first row of phalanges. The muscle fibers continue distal as far as the middle of the carpus, where the muscle becomes wider and thicker. The tendons do not show the split which is later to appear and enclose the deep flexor tendon. The strongest part of the tendons lie on the ulnar side of digits.

The *deep layer* of the preceding stage has undergone marked changes.

The *flexor carpi ulnaris* muscle is quite distinct. It arises partly from the internal condyle superficial to the sublimis and closely connected with it and the palmaris longus and partly from the ulna. The muscle at its origin is broad and thin but narrows into a condensed tissue tendon which is inserted into the os pisiform.

The *flexor digitorum profundus* and the *flexor pollicis longus* muscles arise from the surfaces of the radius and ulna and the internal condyle. They are closely united and pass to the carpal region where division takes place into five well-formed oval tendons, which pass beneath the tendons of the sublimis, and fuse with the condensed tissue about the ends of the digits.

The *pronator quadratus* muscle is a small, oval mass connecting the distal ends of the ulna and radius.

The *lumbricle* muscles are formed. They arise from the profundus near the angles formed by the five tendons. They are short and contain distinct muscle fibers which end in tendons that fuse with the condensed tissue on the radial side of the ulnar four digits.

The intrinsic muscles of the hand, the *interossei*, and muscles of the thumb and little finger, are represented by a late premuscle tissue in which a few muscle fibers are beginning to appear. These masses are more or less continuous with each other and lie on the palmar surface of the carpus and metacarpus and partially in between the latter. The distal ends of these masses fuse with the less differentiated condensed tissue about the digits.

The *extensor* muscles of the forearm show considerable advance over the preceding stage, but the development does not seem to have been as rapid as in the case of the flexor muscles.

Of the *first group*, the *extensor communis digitorum* and the *extensor minimi digiti* are united into a broad, thin sheet which divides in the metacarpal region into four broad, thin tendons that end in the condensed tissue of the four ulnar digits. The *extensor carpi ulnaris* closely associated with this muscle at its origin from the external condyle arises

also partly from the ulna and is inserted into the condensed tissue at the proximal end of the fifth metacarpal. It is quite separate from the common extensor for the greater part of its length.

Of the *second group*, the *brachioradialis* is quite distinct from the *extensor carpi radialis longior et brevior* for most of its length, but at their origin, however, the two are closely connected. Both muscles are broader and larger than in the preceding stage. The extensor passes beneath the third group and ends in the condensed tissue near the proximal ends of the second and third metacarpals.

The *third group*, which arises beneath the first from both radius and ulna, has split more or less into four parts. The proximal one, which is the most completely separated, is the supinator and passes from the ulna and external condyle to the radius. It is united with rest of this group along their ulnar origins, forming thus a continuous sheet for a short distance. The next two pass over the extensor carpi radii tendon, and fuse with the condensed tissue of the first digit. They are the *abductor pollicis longus, extensor pollicis brevis* and the *extensor pollicis longus* muscles. The fourth division is broad and thin and soon joins the deep surface of the tendon of the extensor communis and goes with it to be inserted into the condensed tissue of the second digit.

The Nerves.—By the migration of the arm posteriorly the brachial plexus has been pulled caudally and given a decided posterior inclination. It has also divided into the various cords more than in the preceding stage.

The distribution of the muscle and cutaneous nerves is much as in the adult and as in the next stage.

Embryo XXII.

Embryo XXII measures 20 mm. V. B. and 18 mm. N. B. It is about seven weeks old. The entire arm has a more posterior position. The lower angle of the scapula is at the level of the sixth rib, its anterior limit is about at the seventh cervical vertebra. The entire arm as well as its various parts have increased in size. The muscles are sharper and better developed than the preceding stage. Every muscle that the adult arm presents can now be recognized and each one now contains muscle fibers. The tendons are better formed and can be traced farther towards their final insertions. The ligaments and fasciæ are also more distinct. The process of ligament and tendon formation from the condensed mesenchyma is still in progress at the distal ends of the digits. The skeletal elements are for the most part fairly well formed in cartilage except the distal row of phalanges.

The Skeletal System.—The *vertebral column*. The intervertebral discs are reduced in thickness, while the bodies of the vertebræ have increased and occupy about four-fifths of each segment. The neural and transverse processes are larger and for the most part of cartilage. At the tip of the neural processes, which reach about one-half way around the spinal cord, is a small mass of condensed tissue at what may be considered the growing point. These processes arise entirely from the body and not from the disc. So the body has probably grown at the expense of the disc. The perichondrium, which surrounds the body and its processes, is thickened along the ventral side of the bodies into the anterior common ligament.

The *ribs* are of cartilage surrounded by thick perichondrium, which is continuous with the condensed tissue anlage of the one-half the sternum. The distance between the two halves of the sternum is not as great as in the preceding stage and at the anterior end they are just beginning to come in contact with each other. There are no joint cavities between the ribs and vertebræ.

The *clavicle* is composed of *cartilage* somewhat different in appearance from that in the other bones. It is continuous with the acromion and sternum by an area of condensed tissue. It is surrounded by a typical perichondrium. There are distinct coraco-clavicular, costo-clavicular, and interclavicular ligaments.

The cartilaginous *scapula* is very much larger than in the preceding stage and contains no large areas of condensed tissue. It has moved posteriorly and lies in the region from the last cervical to the fifth thoracic vertebræ. Its dorsal border also extends farther dorsal than in any of the preceding stages. The acromion and coracoid processes are large and of cartilage with only the ordinary thickness of perichondrium which is continuous with that surrounding the rest of the scapula. The spine has not yet appeared but the thickened anterior border from which the supraspinatus muscle arises probably represents by its lateral lip the spine and by its median lip the future anterior border. The acromion arise partially from the lateral side of the anterior border. The head seems to have enlarged at the expense of part of the base of the coracoid process as the long head of the biceps now arises from the junction of the coracoid and the head, and the head of the humerus does not rest against such a large proportional area of the coracoid. There is a distinct suprascapular and a coraco-acromial ligament. At the posterior angle of the scapula there is small mass of condensed tissue which gives attachment to a portion of the serratus, latissimus, and teres major muscles.

The *humerus* is much larger than in embryo XLIII, and has much the adult shape, though of course it is thicker in proportion to its length. It is composed of cartilage. There is a capsular and a coraco-humeral ligament. No joint cavity exists between the scapula and humerus. The tuberosities and condyles are fairly well formed in cartilage and condensed tissue. The bicipital groove is present.

The *ulna* and *radius* are larger and longer than in the preceding stage and are well formed in cartilage. The olecranon, coranoid and styloid processes are partially formed in cartilage and condensed tissue. The perichondrium about the ulna and radius is quite thick. The capsular and orbicular ligaments are present. No joint cavities exist and the cartilages are separated by condensed tissue continuous with the perichondrium.

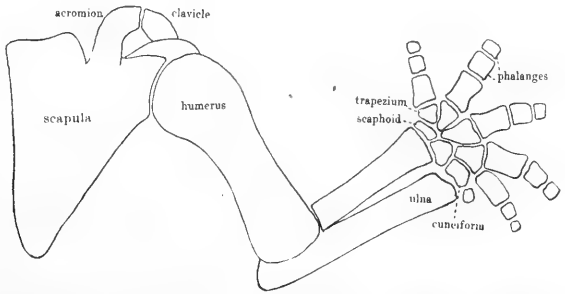

Fig. 13. Cartilaginous skeleton of the arm of embryo XXII, lateral view. × 12 diameters.

All the bones of the *carpus* are represented by cartilage, and in about their relative positions. The amount of condensed tissue matrix is much less than in the preceding stage. The condensed tissue matrix is continuous with the ulna and radius and the five metacarpals without joint cavities. Indications of ligaments of the wrist are present.

The five *metacarpals* are present in cartilage surrounded by thick perichondrium. The first is the shortest.

The first two rows of *phalanges* are present in all the digits. They are of cartilage surrounded by a very thick perichondrium, which is continuous with the condensed tissue between them and the metacarpals and between the phalanges themselves. It is also continuous with the enlarged condensed tissue tip of each digit. There are thickenings for the various ligaments connecting the metacarpals and phalanges and the phalanges with each other.

The Muscular System.—(*Plate II, Fig. C.*) The *trapezius* muscle fibers extend from the occiput to the level of the sixth rib. There is a

considerable interval of fascia connecting them to the neural processes of the lower cervical and the thoracic vertebræ. There is a tendonous attachment to the clavicle and acromion and into fascia or condensed tissue on the surface of the infraspinatus between the trapezius and the deltoid.

The *rhomboid* muscle lies in the region of the seventh cervical to the fourth thoracic vertebræ. It is inserted into the condensed tissue along the dorsal border of the scapula.

The *latissimus dorsi* muscle fibers extend from the humerus to the level of the ninth rib. There is a considerable interval of fascia between them and the neural processes of the lower thoracic and the first two or three lumbar vertebræ. This dorsal fascia is not very well marked. The latissimus also has fibers attached to the condensed tissue at the inferior angle of the scapula.

The *serratus anterior* muscle is separate from the levator scapulæ except near its attachment to the scapula. It is a broad, thin sheet, having digitations to the first eight ribs.

The *pectoralis major* muscle is well developed. The separation between the clavicular and the sterno-costal portions is less marked than in the preceding stage. The muscle is attached as low as the sixth rib.

The *pectoralis minor* muscle is quite distinct from the major, as a considerable layer of loose mesenchymal tissue lies between them. It arises from the second, third and fourth ribs and passes to the coracoid process.

The *subclavius* muscle is inserted into the clavicle at an angle of 45°. As the scapula and clavicle sink down towards the level of the first rib the angle at which this muscle is inserted into the clavicle decreases.

The *teres major* muscle arises from the lower angle of the scapula and passes to the humerus. It is interesting to note that at this stage tendon of the latissimus dorsi twists around the lower border of the teres to be inserted with it into the humerus.

The *deltoid* muscle is large and well developed.

The *supraspinatus* muscle arises from the thickened anterior border of the scapula. It cannot be said to take origin more from the lateral surface than from the median surface of the scapula.

The *infraspinatus* muscle occupies the middle of the lateral surface of the scapula and passes beneath the deltoid to the great tuberosity of the humerus.

The *subscapularis* muscle arises from most of the median surface of

the scapula. Its tendon of insertion is broad and thin and closely applied to the capsular ligament.

The three heads of the *triceps* muscle are easily distinguished. The long and external heads are of about the same size. The *anconeus* muscle is continuous with the triceps but arises from the external condyle and passes to the side of the olecranon and adjoining surface of the shaft of the ulna.

The long head of the *biceps* muscle arises from the junction of the coracoid process and the head of the scapula and passes through the bicipital groove. The two heads are inserted together into the condensed tissue swelling on the radius.

The *coracobrachialis* muscle and short head of the biceps are intimately connected for most of the length of the former.

The *brachialis* muscle has spread out over more of the distal surface of the humerus than in the preceding stage.

The *flexor* muscles of the forearm are easier to distinguish than in the preceding stage.

The tendon of the *palmaris longus* is narrower in proportion than in embryo XLIII.

The tendon of the *flexor carpi radialis* muscle can be traced farther towards its insertion into the base of the second metacarpal than in embryo XLIII.

The muscle fibers of the *flexor sublimis digitorum* still run to the carpus before the wide tendon begins. This tendon soon splits into four tendons which go to the four ulnar digits. These tendons are better formed than in the preceding stage and split to surround the tendons of the deep flexor. Their ends fuse with the thick perichondrium about the phalanges.

The *flexor carpi ulnaris* muscle shows distinctly its two heads of origin. It has a well-formed tendon of insertion.

The *deep flexor* muscles can be separated into the *flexor pollicis longus* and the *flexor profundus digitorum* muscles. The muscle fibers of the profundus continue into the carpal region and end in a broad tendon which divides at the base of the metacarpus into four well-formed tendons. These fuse with the condensed tissue at the tips of the digits. There is a slight split in each of these tendons near its end. The tendon of the flexor longus pollicis behaves similarly.

The *pronator quadratus* muscle is oval in cross section, and connects the distal ends of the radius and ulna.

The *lumbricales* are quite well developed and their fairly well-formed tendons end in the perichondrium on the radial side of the digit.

The *interossei* muscles and the small muscles of the thumb and little finger are now fairly well developed. Muscle fibers are present.

The *extensor* muscles of the forearm show considerable advance over the preceding stage. The tendons of the *extensor communis digitorum* are longer and narrower. The muscle fibers continue to the base of the metacarpus, where the splitting into the four tendons takes place. The tendons are inserted into the condensed tissue tips of the digits. The edge of the tendons near their insertions are more or less continuous with the perichondrium about the digit.

The tendon of the *extensor carpi ulnaris* is beginning to form. One branch of it seems to join the communis tendon. This may be the tendon of the extensor minimi digiti.

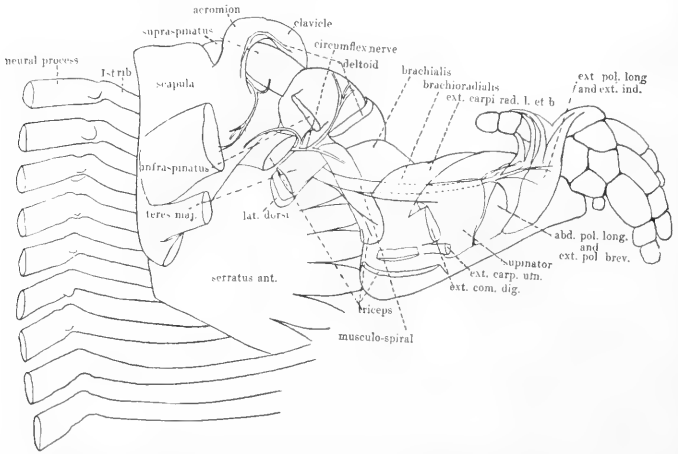

FIG. 14. Lateral view of the arm of embryo XXII, from Plate VIII. Bardeen and Lewis, Vol. I, this Journal. × 12 dia.

The *extensor carpi radialis longior et brevior* are not to be separated.

The *supinator* muscle is well developed and has the posterior interosseus nerve passing through it.

The *abductor pollicis longus* and extensor pollicis brevis muscles are only to be separated where the muscle fibres pass into tendons, which fuse with the perichondrium of the first digit. The separation occurs at the lower end of the radius. These two muscles are fairly distinct from the supinator and the *extensor pollicis longus* and *extensor indicis proprius* muscles. The last two muscles are inseparable for part of their course and shortly after dividing each forms a round tendon. The extensor pollicis longus then spreads out into the sheath about the first

digit. The extensor indicis muscle joins the ulnar side of the tendon of the communis to the second digit.

The Nerves.—The *brachial plexus* has a decided posterior inclination and seems to have been pulled down against the first rib. The three cords are so close together that it was impossible to separate them satisfactorily though indications of the cords are present. There is nothing especially peculiar about the distribution of the nerves from the plexus, either motor or sensory, which is not present in the adult.

Summary.

The first indications of the arm bud appear during the third week as a slight swelling in the lower cervical region on the anterior portion of the Wolffian ridge. This gradually enlarges and by the time the embryo is 4.5 mm. in length and three weeks old the arm is of considerable size. The base now lies opposite the lower four cervical and first thoracic vertebræ. The arm bud is at first filled with a homogeneous and closely packed mesenchyma. No nerves or myotome buds have entered the arm, yet it contains the tissue from which the muscular and skeletal elements develop.

During the fourth week before the nerves enter differentiation begins by an increased condensation for the skeletal core. The nerves, however, have reached the base of the arm and have united by their expanded ends into the first beginnings of the cervico-brachial plexus. During the fifth week the nerves from this plexus push into the premuscle sheath which surrounds the skeletal core.

By the end of the fifth week the skeletal core can be differentiated into many of the skeletal elements, three of which contain cartilage, namely, the humerus, ulna and radius. The premuscle sheath also has become more or less differentiated into muscles or groups of muscles, between which, however, no sharp lines can, as a rule, be drawn. Toward the distal end the differentiation is less complete, and in the hand premuscle tissue still represents the intrinsic muscles. The nerves have grown into the hand and spread out in a very peculiar manner. Most of the branches of the brachial plexus found in the adult are now present.

By the end of the sixth week most of the muscles of the arm are easily recognized. The intrinsic muscles of the hand are just beginning to show fibrillation and are still mostly of premuscle tissue. The tendons and ligaments are also becoming more sharply differentiated. Most of the skeletal elements consist of cartilage and the surrounding

thick perichondrium. The clavicle, some of the carpals and the second row of phalanges are of condensed tissue, while the distal row of phalanges are not differentiated as yet. The nerves, both sensory and motor, are distributed much as in the adult.

By the end of the seventh week all the skeletal elements are of cartilage except the distal row of phalanges from the second to fifth digits, which are of condensed tissue. All the muscles are to be recognized and are composed of muscle fibers. The tendons and ligaments, except in the distal part of the digits, are well formed. The digits present a very interesting picture of the differentiation of the cartilage, perichondrium, ligaments, and tendons from the condensed tissue tip of each.

During the process of differentiation other important changes are taking place, namely, the migration caudally of the whole arm, the migration or extension of certain muscles from the arm caudally along the body wall and the migration of other muscles from more anterior regions to the arm, shoulder girdle and thorax.

We may consider the position of the scapula and the inclination of the brachial plexus as indicators of the migration of the arm. We find, in an embryo of four and one-half weeks, that the scapula lies in the region of the fourth and fifth cervical vertebræ. The brachial plexus and the nerves forming it run to the arm without any caudal inclination. The nerves which leave the plexus do bend posteriorly in the arm. At five weeks the scapula has greatly enlarged and extends from the fourth cervical to the first dorsal vertebræ. Its center has evidently shifted posteriorly. The brachial plexus and the anterior nerves which go to it have a slight caudal inclination. By the end of the sixth week the greater portion of the scapula lies below the level of the first rib, its posterior angle, including the condensed tissue, having extended to the level of the fifth rib. The brachial plexus has been pulled along with the shifting of the arm and has a decided posterior inclination. By the end of the seventh week very little of the scapula lies above the level of the first rib, and its lower angle reaches to the fifth intercostal space. The brachial plexus has a very marked caudal inclination and appears to be bent over the first rib. Before the adult conditions are attained the scapula must migrate some distance posteriorly. Part of this movement will take place with the sinking posteriorly of the ventral portion of the thoracic wall, for in these stages the ventral ends of the ribs are as far anterior as the vertebræ from which they arise.

The migration of the pectoralis major and minor and the latissimus dorsi muscles from the arm posteriorly to the thoracic wall is very

evident from the stages we have studied. At a very early stage these masses receive their nerves and later drag them posteriorly. By the seventh week the pectoral muscles have reached their adult positions so far as the thoracic attachments are concerned. The latissimus dorsi, even by the end of this week, only extends to the ninth rib.

Another very important group of muscles migrate from the head and anterior cervical region to the arm and thorax. In an embryo, four and one-half weeks old, the posterior end of the trapezius premuscle mass lies at the level of the fourth cervical vertebra, at five weeks the muscle fibers extend to the level of the fifth cervical vertebra, and at six weeks to the fifth thoracic vertebra. At this age also the muscle has acquired its attachment to the scapula and clavicle. At seven weeks the muscle extends to the level of the sixth thoracic vertebra. The spinal accessory nerve is connected to the premuscle mass as early as the middle of the fifth week, and as the muscle extends posteriorly the nerve is carried along with it.

The sterno-mastoid muscle originates high up in the neck with the trapezius. It extends posteriorly and ventrally, reaching the clavicle and sternum during the sixth week.

The infrahyoid muscles also migrate from the anterior neck region, carrying their nerves down with them.

The rhomboid premuscle mass at 4.5 weeks lies at the level of the fifth cervical vertebra and gets its nerve supply at this time from the fifth cervical nerve. At five weeks it has extended to the sixth cervical vertebra, and at seven weeks it is for the most part in the thoracic region and has acquired its scapular attachment.

The serratus anterior premuscle mass at four and one-half weeks already extends into the upper thoracic region and has its cervical nerve supply. It has probably already migrated from the cervical region. At five weeks it has reached its posterior attachment on the thorax, but is not as yet attached to the scapula. This occurs during the sixth week. The serratus anterior muscle is thus one of the first of these migrating muscles to attain its permanent attachments. It is also evident that the various serrations of this muscle are of secondary origin.

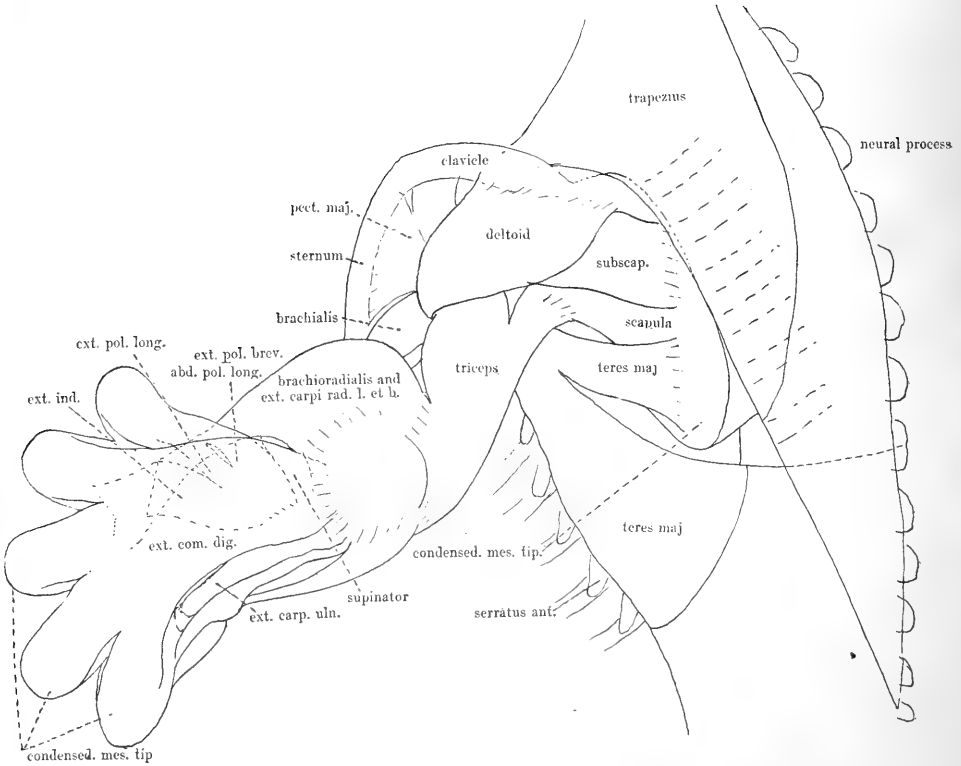

PLATE I, FIG. A. Lateral view of the arm region of embryo XLIII. × 20 diameters.

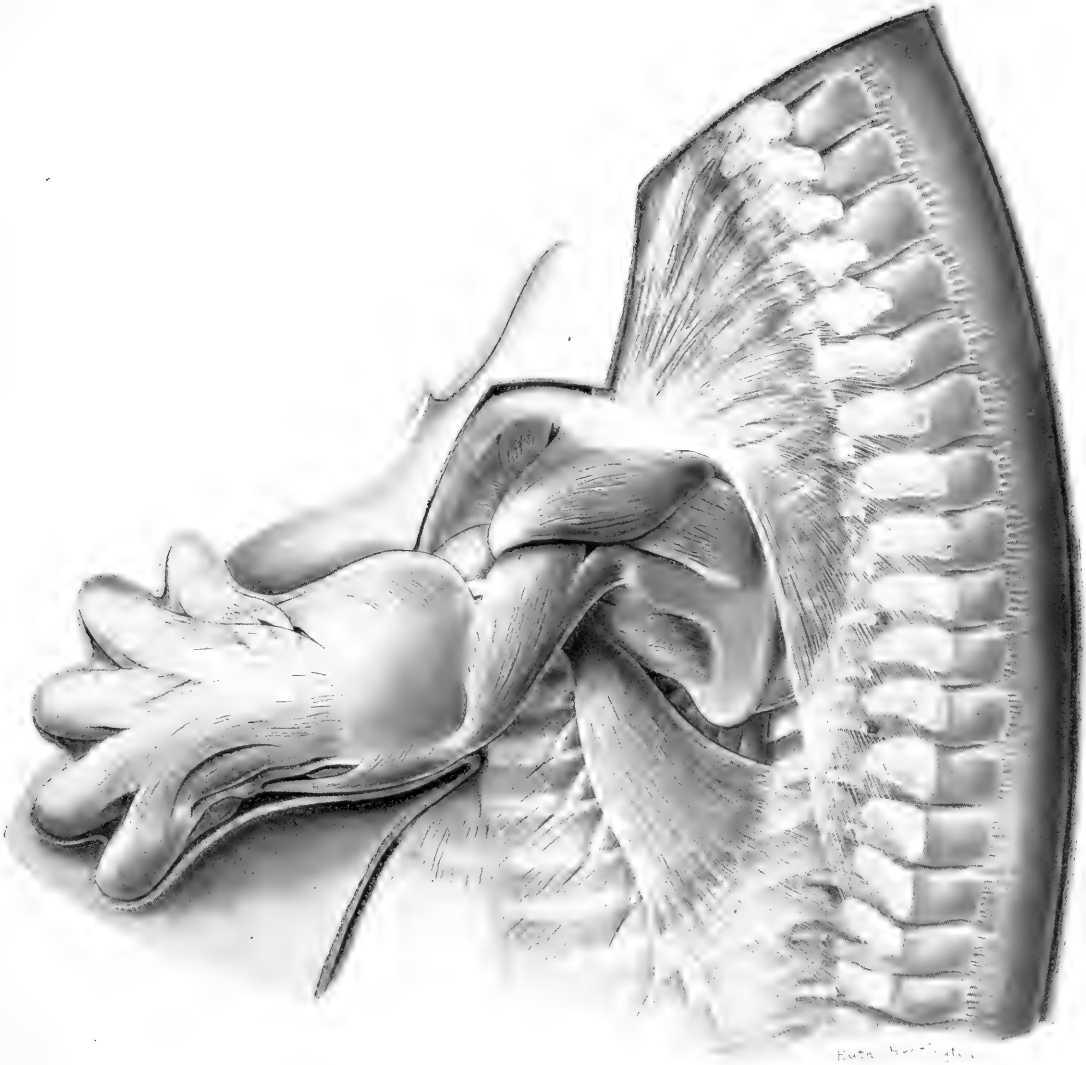

FIG. A.

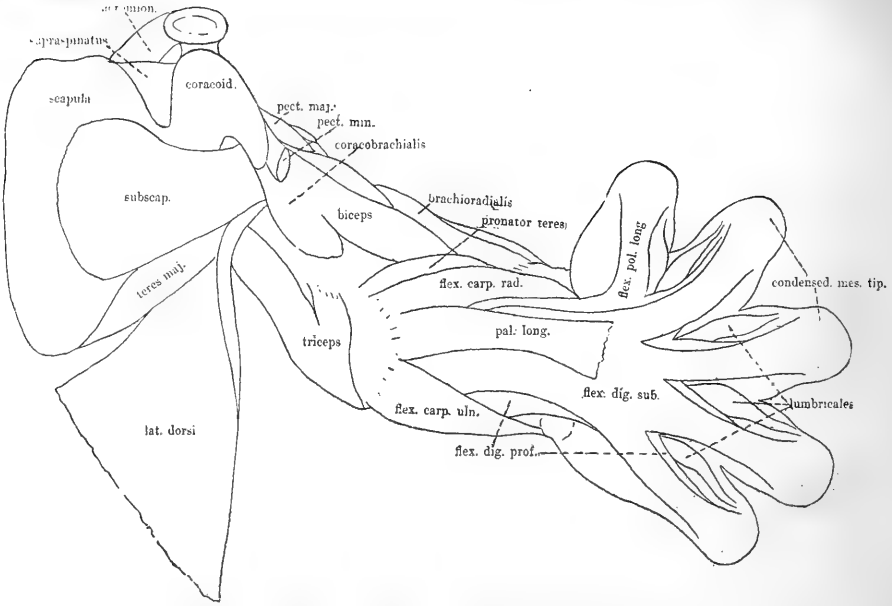

PLATE II, FIG. B. Median view of left arm of embryo XLIII. × 20 diameters.

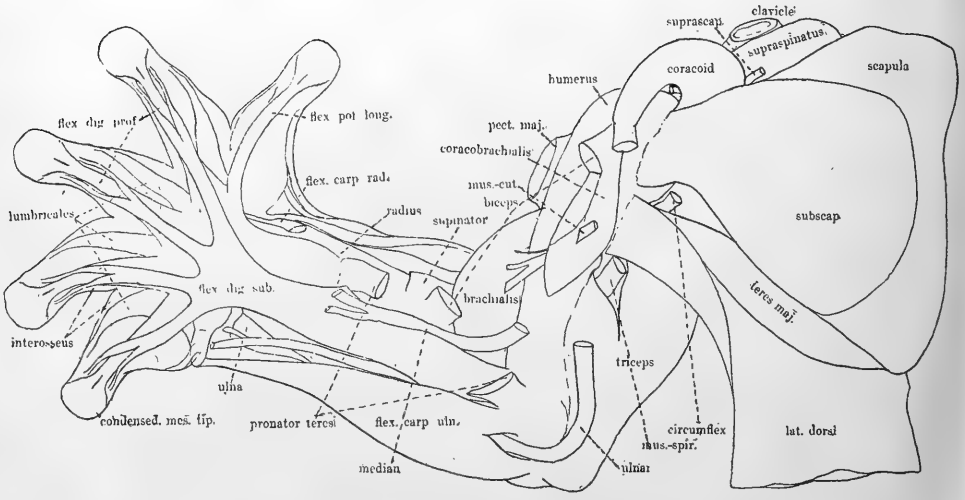

PLATE II, FIG. C. Median view of right arm of embryo XXII. × 20 diameters.

FIG. B.

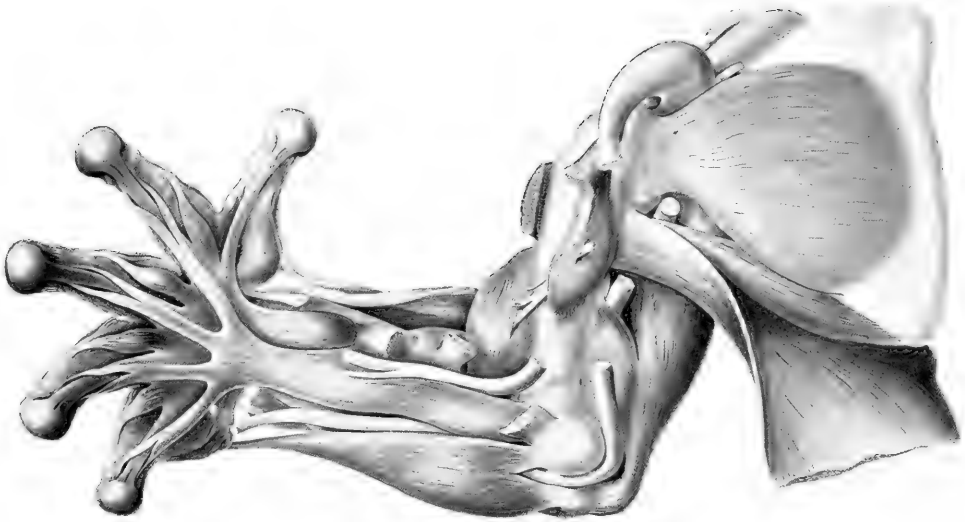

FIG C.

THE DEVELOPMENT OF THE EYE MUSCLES IN ACANTHIAS.[1]

BY

ARTHUR B. LAMB.

From the Biological Laboratories of Tufts College.

WITH 9 TEXT FIGURES.

These studies were undertaken at the suggestion of Dr. J. S. Kingsley and were carried on during the year 1899-1900 under his direction, at the Biological Laboratory of Tufts College.

My specimens were killed either in aqueous corrosive sublimate or in Davidoff's corrosive-acetic mixture. Delafield's hæmatoxylin was principally used as a staining agent. Wax reconstructions were made according to the method of Born. My results are largely but confirmatory of those of Van Wijhe, Miss Julia Platt, Hoffmann and Neal. It is hoped, however, that a presentation of the subject freed from externals may, together with the series of reconstructions submitted, assist in the comprehension of the process of development.

The discovery that in selachii the eye muscles are developed from the epithelial walls of the 1st, 2nd and 3rd head somites was made by Marshall. His results have been repeatedly confirmed, and there can be no reasonable doubt of their validity. I use the term "somite" advisedly, being convinced that in Acanthias the head cavities are comparable with trunk somites. Neal, **98**, p. 187, presents an excellent summary of the evidence on this point. A history of the development of the eye muscles is therefore a history of the origin and differentiation of these head somites. At this point I wish to call attention to the very detailed account of the early history of these somites, presented by Hoffmann, **96**, in his "Embryology of the Selachii."

ANTERIOR SOMITE.—Before passing to a consideration of the eye muscle somites proper, I propose, for the sake of completeness, to consider the anterior somite. Van Wijhe saw (**82**, p. 13), in the single specimen of Galeus at his disposal, on either side of the head, anterior to the 1st or premandibular somite, a slender cavity with distinct and

[1] Studies from the Biological Laboratories of Tufts College, under the direction of J. S. Kingsley, No. XXIX.

thickened walls. He homologised this with the anterior prolongation of the first somite observed by him in Pristiurus and Scyllium. He therefore considered it as merely a secondary subdivision of that cavity.

Miss Platt, 91, observed similar cavities in Acanthias, and to her is due the name of "anterior head cavities"; it seeming unwise to alter the numbering of the remaining head somites for the sake of a pair of somites which are only known to occur in two forms. It also de-

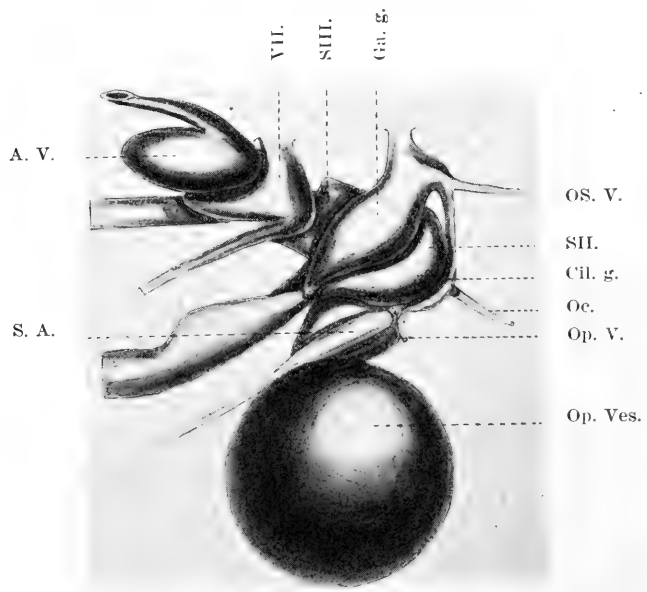

FIG. 1.—Reconstruction of auditory and optic vesicles, ganglia of fifth and seventh nerves and the anterior, first, second and third somites of the right side of an Acanthias embryo, 12½ mm. total length. Lateral view.

serves mention that Zimmermann, 91, p. 113, recognized, in the same year and quite independently of Miss Platt, the presence in Acanthias, of this somite. According to Miss Platt, the archenteron, which extended forward to the anterior neuropore as a solid mass of cells, was divided into an anterior and a posterior part by the down-growing infundibulum. Both parts grew laterally; the anterior forming the anterior head somite, the posterior the 1st or premandibular somite. Hoffmann was able to add to this the observation, that the down-growing *anlage* of the infundibulum not only divides this process of the archenteron into an anterior and a posterior part, but that it also sub-

divides the anterior part into three smaller portions: one axial in position, two lateral and paired. From these lateral portions develops, on either side, the anterior head cavity of Miss Platt. Of the axial portion as much as lies beneath the infundibulum is aborted, while its anterior part persists and forms a connecting stalk by which the somite of one side is joined with that of the other. This connecting stalk is similar to that (*C. st.* in figures) which joins the first (premandibular) somite of one side with that of the other. The only difference is that there we have a canal, while here we have a loose and solid strand of

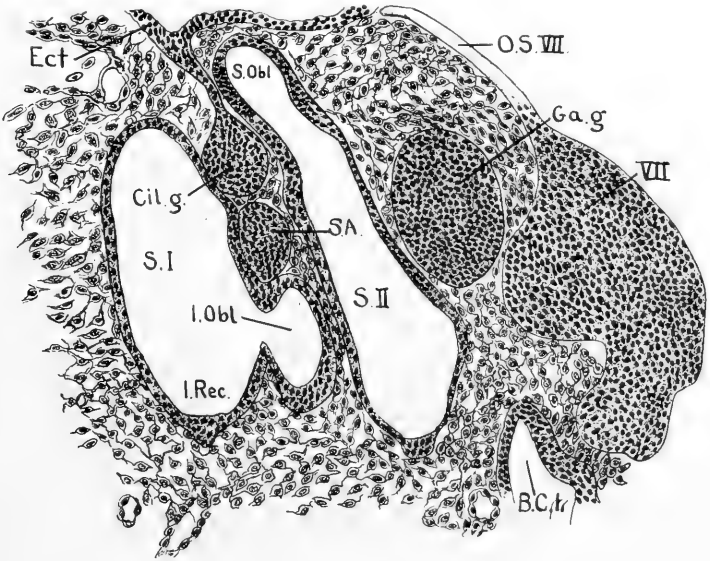

FIG. 2.—Parasagittal section of somites A, I and II of Acanthias embryo of 16 mm. total length showing the early differentiation of muscles inferior and superior obliquus and inferior rectus.

connective cells. It will be seen that Hoffmann differs from Miss Platt, simply in the recognition of an axial portion which later gives rise to a connecting strand. Stages in which 29-33 somites were differentiated showed very clearly the presence of this axial portion as described and figured by Hoffmann. See also Minot's figures (01, pp. 82, 83).

At a 32 segment stage the somite is elongate in form, its main axis extending obliquely downward beneath the eyeball. It is smaller at its dorsal end, where the cells have assumed a somewhat epithelial arrangement, and a slight lumen is present. Ventrally, the somite consists of a

large mass of cells rather loosely packed, and occupying the space between eye-ball and epidermis. The somite is pressed closely against the anterior walls of both the 1st and the overlapping 2nd somite.

As the embryo grows older the somite assumes a much more elongate form (Fig. 1). The lumen increases in size and extends far into the ventral process, while the walls become considerably thinner and but one cell in thickness; still, they never become so thin as those of succeeding head somites. At a fourteen mm. stage the walls have again become thickened. This is especially true of the median and posterior walls. At a later stage (16 mm.) the cells are being freely proliferated into the lumen of the somite, and the median thickening is very marked. This proliferation continues, probably externally as well as internally, for the outline of the somite becomes gradually indistinct. At a 19 mm. stage (Fig. 4, *SA*) the somite consists simply of a solid mass of cells, gradually thinning out into the general mesenchymatous tissue. At a 26 mm. stage no trace of the somite can be seen. While, as Hoffmann says, no muscle fibres are formed by this somite, still, as both Miss Platt, **91**, and Neal, **98**, observed, the cells proliferated into the cavity assume an elongate form.

The anterior somite undergoes an interesting change in its position relative to the first somite. Originally pressed against the anterior walls of both the 1st and 2nd somites it comes, at a 12.5 mm. stage (Fig. 1), to occupy a position lateral to somite I, and in the angle between that somite and somite II. This seems to be due to the great enlargement of the eye vesicle, and also to the forward growth of somite I. Soon, however, the outpocketing from the posterior end of somite I, which gives rise to the inferior oblique, appears, and this ultimately grows nearly around the anterior somite, so that this later somite occupies a deep depression in its wall (Figs. 2 and 3).

Neal, **98**, p. 227, found at a 65 segment stage processes apparently extending from the ciliary ganglion to this somite. I have not been able to find such processes of whose nervous character I was certain.

First, or Premandibular Somite.—The epithelial walls of this somite give rise to four of the six muscles of the adult eye. It is therefore preeminently the eye-muscle somite of the head. Balfour stated that this cavity was cut off from the anterior end of the cœlom by the formation of the first gill cleft. Marshall also held that it was cut off from the anterior end of the cœlom, but that this took place independently of the formation of the gill cleft. In Scyllium and Pristiurus, Van Wijhe found that the cavity was never in other than potential connection with the primary cœlom, arising independently of it

from the undifferentiated mass of cells in which the notochord anteriorly ends.

In Acanthias, as was shown above in the history of the anterior somite, the down-growing infundibulum divides the anterior prolongation of the archenteron into an anterior and posterior portion. From the anterior portion the anterior somite develops. The posterior por-

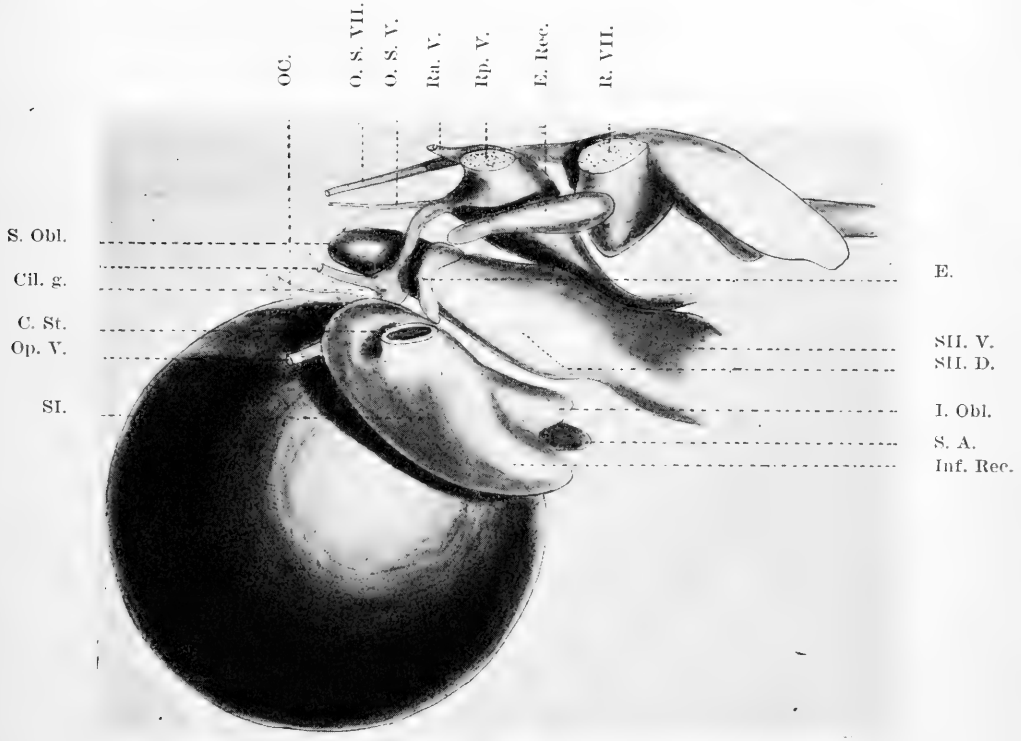

FIG. 3.—Reconstruction of optic vesicle, head somites and nerves of an Acanthias embryo, 16 mm. total length. Right side, medial view.

tion, growing laterally, gives rise to the first somite of either side, while axially it forms the connecting stalk or canal so characteristic of this somite (Fig. 3, *C. st.*). At a stage when 21-22 somites are differentiated, both the somite and the connecting strand are solid. This is still the case at a 29-30 mm. stage, except that in the connecting stalk a small cavity is visible. At a slightly later stage the number of these median cavities has increased. Miss Platt, 91, homologised these with the median cavities described by Dohrn, 90, in Torpedo.

A considerable lumen can now be distinguished in the lateral somites. The median stalk is continuous at its middle with the notochord above and the alimentary canal behind. Hoffmann describes three processes arising at this stage (32 segments) from the 1st somite:

I. A process extending backwards and downwards, running close to, and parallel with, the visceral prolongation of the 2nd somite. He considered it probable that this process, although he had never seen a lumen in it, was comparable to the hollow process found by Zimmermann, 91, in Pristiurus, connecting the premandibular somite with the ventral cœlom. Neal, 98, p. 201 note, while confirming the presence of this "Zellstrang," was very certain that it was derived not from the mesoderm but from the neural crest, having followed the migration of neural crest cells ventrally into the mandibular arch. Neal also called attention to a similar strand of cells situated *posterior* to the visceral portion of the mandibular cavity. While I found that this strand of cells is apparently continuous with a slight outgrowth from the 1st somite, as figured by Hoffmann, this continuity seems to be more apparent than real. In the first place, I am able to confirm the presence of the posterior strand described by Neal. Further, I found this posterior strand not only continuous laterally with the anterior strand, but dorsally with neural crest cells. This is especially evident at a 32-33 somite stage. I therefore conclude with Neal that this "Zellstrang" is not a process of the somite, but is rather derived from the neural crest.

II. A process extending ventrally forward below the anterior cavity. A similar process was seen by Van Wijhe in Pristiurus and was homologised by him with the anterior head cavity of Galeus. Since the anterior cavity in Galeus is in all probability homologous with that in Acanthias, if Hoffmann's process in Acanthias is homologous with that found by Van Wijhe in Pristiurus, then, since both occur at once in Acanthias, the homology drawn by Van Wijhe could not be maintained. Hoffmann believed this to be the case.

I have been able to find but very slight evidences of this process. I am led therefore to doubt the homology drawn by Hoffmann between this process and the process described by Van Wijhe in Pristiurus. Consequently, I cannot on this ground take exception to the homology drawn by Van Wijhe between the process of the 1st somite in Pristiurus and the anterior somite in Galeus.

III. A process extending dorsally along the anterior surface of the 2nd somite. It is small in extent and transitory in appearance. I am inclined, here as before, to doubt the real continuity between this

process and the somite, since it seems to me that it might equally well be derived from neural crest cells.

As the embryo develops, the median stalk is pushed away from the alimentary canal and around the end of the notochord by the intervening aorta. The notochord thus shifts its position from the dorsal wall of the median stalk to its posterior wall. Such is the compression

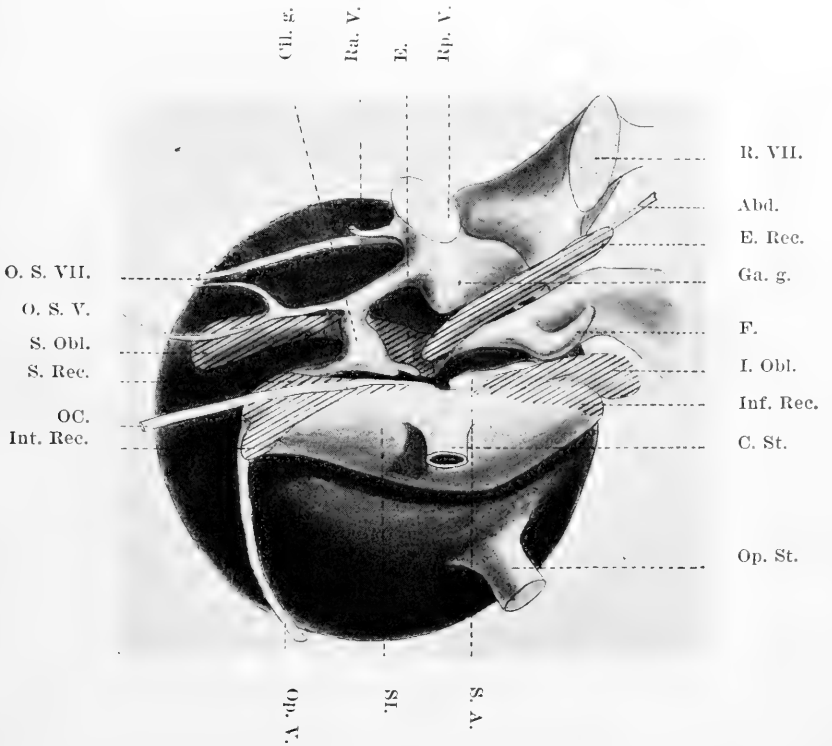

FIG. 4.—Reconstruction of optic vesicle, ganglia and somites of an Acanthias embryo, 19 mm. total length. Right side, medial view. The *Anlagen* of the muscles lined obliquely.

that exists in this region that the median stalk is nearly severed at the point of contact. The irregularly placed median cavities of the stalk now fuse together and finally assume connection with the lumen of the somite on either side. The lumen of the median stalk persists until a late stage, when the walls become mesenchymatous and the cavity is obliterated. The stalk persists, however, as connecting strand until nearly a 30 mm. stage (*C. st.* in Figs. 1-7).

The thickenings of the epithelial walls which give rise to the four muscles, occur in general on the dorso-median walls. The first thickening appears on the more ventral end of the somite. This soon becomes a large outpocketing with thick walls (Fig. 3, *I. Obl.*). It is the anlage of the muscle obliquus inferior. This therefore is, as Hoffmann pointed out, the first of the oculomotor muscles to be differentiated.

The next thickening to appear is located very near the above-mentioned *anlage* of the inferior oblique. It gives rise to the muscle rectus inferior (Fig. 3, *Inf. Rec.*). At a later stage thickenings appear on the more dorsal end of the somite. These do not become well marked and differentiated from one another until a somewhat late stage, about 20-22 mm. The more dorsal of these thickenings forms the muscle rectus superior; the more ventral, the muscle rectus interior.

It will be seen from the above that the muscles arise in two pairs, one at either end of the somite.

The outpocketing which is to form the inferior oblique soon becomes constricted off from the somite. At a 19 mm. stage its lumen has nearly disappeared, and the muscle has assumed an elongate form (Fig. 3). The direction of the principal axis as well as the direction of its muscle fibres is longitudinal. In the adult the direction is also nearly longitudinal, but it will be seen from the series of reconstructions (Figs. 4, 6, 7, 8), that the originally anterior end has become posterior; i. e., the direction of the muscle is nearly reversed. This transformation is brought about by a revolution of the posterior end about the anterior end as a centre, combined with a general ventral shifting of the muscle. The adult condition is approximately reached at a 33 mm. stage (Fig. 8).

The thickening, which is to form the inferior rectus, and belonging to the same pair as the inferior oblique, at first extends parallel to that muscle and therefore in a longitudinal direction (Fig. 4, *Inf. Rec.*). At a 26 mm. stage it has turned through approximately a right angle, and runs in a general dorso-ventral direction (Figs. 6, 7, *Inf. Rec.*).

The thickenings which arise in the more anterior and dorsal end of the somite, and which give rise to the superior and internal recti, have only become clearly differentiated at a 25 mm. stage (Fig. 7). The internal rectus retains its nearly longitudinal direction; the superior rectus describes approximately a right angle about its posterior end. At a 33 mm. stage (Fig. 8) it has approximately reached its adult dorso-ventral direction. Except where the muscle thickenings have been formed, the walls of the somite retain their single layered epithelial character until about a 27-30 mm. stage, when they become converted into loose mesenchyme and the outline of the somite is lost.

The musculature arising from this somite is innervated by the oculomotor. This nerve is differentiated at an 8 mm. stage. It arises, Neal, 98, from the ventral floor of the mid-brain, as processes from neuroblast cells in the ventral horn of this encephalomere. It extends backward to the ciliary ganglion and runs through it to the walls of the 1st somite. Neal, 98, p. 227, found, at a stage before the appearance of the oculomotor, processes extending from the ciliary ganglion to the somite, similar to those found in connection with the anterior

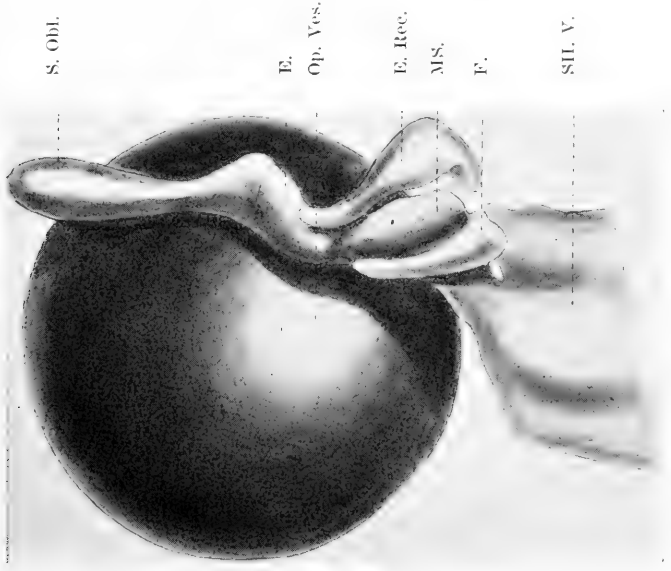

Fig. 5.—Reconstruction of optic vesicle and derivatives of the mandibular and hyoid somites of the right side of an Acanthias embryo, 22 mm. long, viewed from medial side.

somite. As in that case, I have been unable to convince myself that the fibres which seem to connect ganglion and somite are really nervous in character.

Second, or Mandibular Somite.—This somite is the largest in the head, and is characterized by the possession of a visceral portion connecting it with the ventral cœlom.

Hoffmann, 96, found this somite marked off by constrictions from the general body cavity at a 20 somite stage. A contracted lumen was present. At this stage the floor of the brain is pressed closely down upon the notochord and somite, but as growth takes place it draws

away, and a considerable space is left beneath it. Into this space a proliferation of mesenchyme cells takes place from the anterior-median wall of the somite.

The walls of the somite at this stage are thin and single-layered, except in the median side, where the cells are higher and a tendency towards the formation of two layers is evident. Later, the median wall becomes thicker, and the area of mesenchyme proliferation more definite. The cells derived from this outgrowth are spreading out around the walls of the somite. This outgrowth seems to me to be

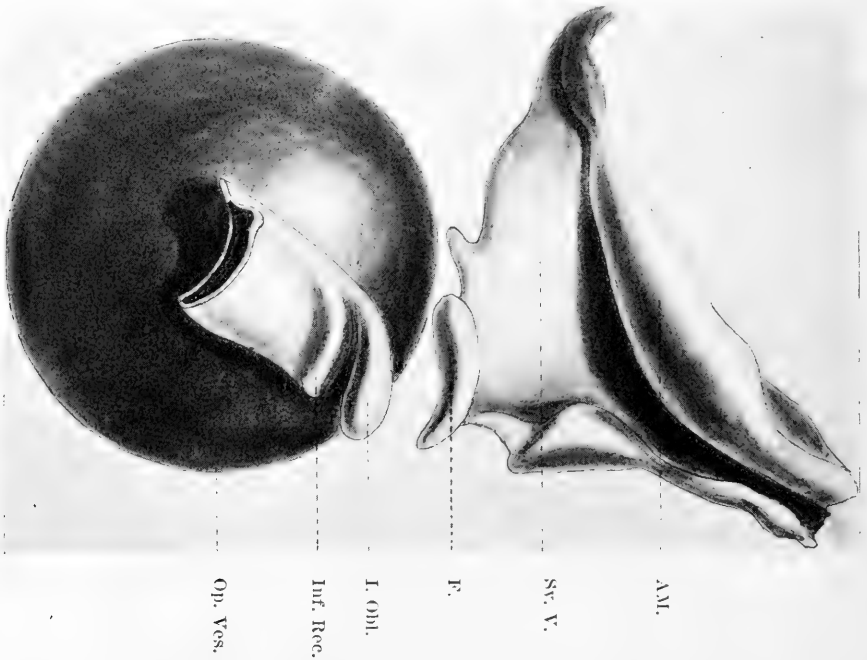

FIG. 6.—Reconstruction of optic vesicle and derivatives of the premandibular somite of an Acanthias embryo, 26 mm. total length. Right half, medial view.

comparable to a sclerotome of a trunk somite, both in position and in histological appearance.

At a 10 mm. stage the lateral, as well as the posterior median walls, have become very thin. This thinning out at posterior median wall continues, and soon the epithelial character of the bounding tissue is lost. This break, together with a constriction which gradually takes place, divides the somite at this point ultimately into a dorsal and a

visceral part. At a 12 mm. stage the lumen of the one part is completely separated from that of the other. Until a late stage, however (24 mm.), the two parts are connected by strands of mesenchymatous tissue (Fig. 5, *MS.*).

For the sake of clearness I will treat the further development of dorsal and visceral parts separately.

Dorsal Part, or the Myotome Proper.—At a fourteen mm. stage a large outpocketing with slightly thickened walls is apparent at the anterior end of this part of the somite. This outpocketing is the anlage of the muscle obliquus superior. At the base of this outpocketing, on the median side of the somite, a thickening of the epithelium is evident. This is the anlage of a rudimentary muscle first mentioned by Miss Platt, and spoken of by her as "Muscle E." The walls of the remainder of the dorsal part are very thin.

Sixteen mm. stage (Fig. 3). The outpocketing giving rise to the superior oblique has become very thick-walled. "Muscle E" is well developed. The direction of its principal axis, as well as of its fibres, is longitudinal. It is not straight, however, but its anterior end is curved outwards.

From this stage on, those parts of the walls not forming muscles rapidly degenerate, and the lumen of the somite is usurped by mesenchymatous tissue. A contracted lumen, however, persists until a late stage in the anlage of the superior oblique muscle. The general direction of this muscle and of the fibres which are now present in it, is longitudinal. The whole muscle migrates forward, the posterior end becoming attached, while the anterior end moves ventrally. The muscle consequently comes to extend in a dorso-ventral direction (Figs. 4, 5, 8, *S. Obl.*). At a 19 mm. stage (Fig. 4) it is still longitudinal and still retains connection with "Muscle E" by a strand of connective tissue. This latter muscle has now reached its maximum development. The anterior end curves not only outward but upward as well, so that the direction of the muscle is approximately dorso-ventral. From now on this muscle undergoes degeneration. At a 26 mm. stage scarcely a trace of it remains.

Visceral Part.—At a 12 mm. stage the walls of this part, except where they are continuous dorsally with the somite proper, are closely compressed right and left. At the ventral end the walls are very thick, and constitute the *anlage* of the muscle adductor maxillæ.

At a 14 mm. stage an outpocketing appears at the dorsal end. This is the *anlage* of a rudimentary muscle which Miss Platt, 91, first recognized. Hoffmann, 96, considered this muscle identical with that de-

scribed by Vetter, 74, and designated by him, muscle levator labii superioris. He does not, however, give any reasons for this view. Miss Platt was able to trace this muscle only until it came to occupy a position in close proximity to the inferior oblique eye muscle. She believed, however, that the muscle was permanent.

This outpocketing, as will be seen in the reconstructions, can readily be followed until a 26 mm. stage (Figs. 4, 5, 6, *F*). At a 22 mm. stage

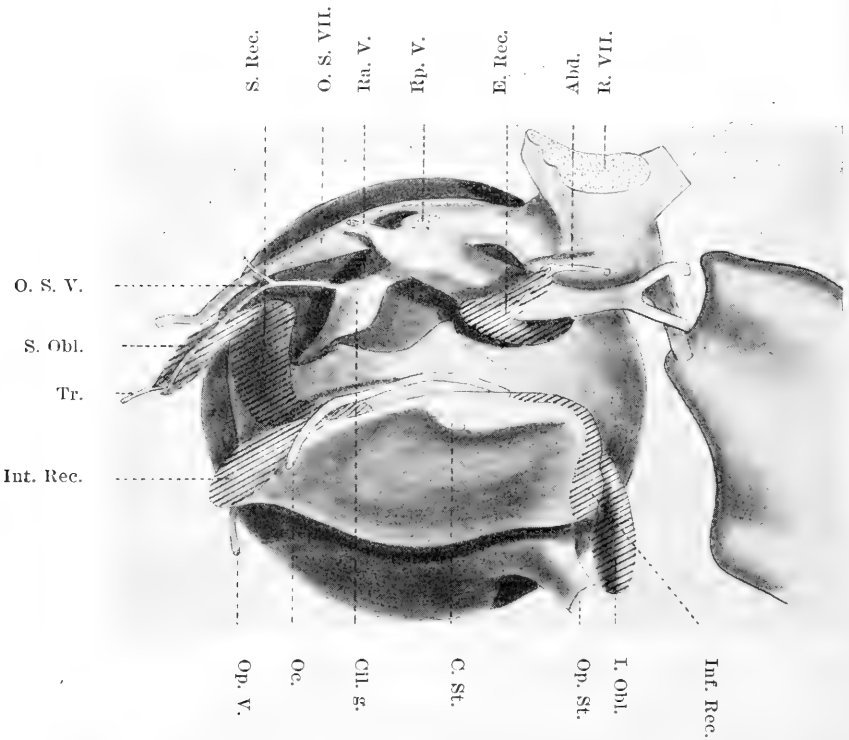

FIG. 7.—Optic vesicle, nerves and developing eye muscles of an Acanthias embryo, 27 mm. total length. Right side, medial view. The oblique lines indicate the parts of the somites which are being converted into muscles.

its walls are thin and enclose an extensive lumen. At a 26 mm. stage its lumen has disappeared and its constituent cells have become elongate and apparently muscular. The muscle is situated at this stage, as Miss Platt stated, close to the inferior oblique eye muscle. As will be seen in reconstructions, it is nearly continuous with the thickened ventral edge of the remainder of the visceral part. The cells of this

thickened edge seem also muscular. At a 27 mm. stage I have been able to find only the very slightest remains of this muscle. Such a rapid degeneration seems, however, improbable, and requires, I feel, further investigation.

The superior oblique muscle derived from this somite is innervated in the adult by the trochlearis. This nerve, however, is the last cranial nerve to be differentiated, not appearing until a 21-22 mm. stage (Fig. 5). At a 16 mm. stage, as several investigators have shown, the small ramus ophthalmicus superficialis V. sends fibres to this somite. From this it might be inferred that motor impulses were originally transmitted to this somite by the 5th nerve. Neal considers this supposition untenable, since in embryos of but 19 mm. length, consequently before the appearance of the trochlearis, the ramus ophthalmicus superioris V. shows no connection with the muscle. While unwilling to contradict this latter statement and say that such a connection does exist, I should be even more unwilling, because of the *very* close proximity of muscle and nerve at 19-24 mm. stages, to say that such a connection does *not* exist.

Third, or Hyoid Somite.—This is the most posterior somite which contributes to the musculature of the eye, giving rise to the external rectus muscle. It is marked off from the rest of the body cavity merely by constrictions before and behind, at a time when the 7th somite of Van Wijhe is completely separated. This emphasizes the progressive development which takes place both forward and backward from a point in the neck region. At this stage its form and position is very similar to that of the four succeeding head somites. At a 22 segment stage a contracted lumen is generally visible. Neal points out that at this stage the somite is in every way comparable with a trunk myotome. It is plainly dorsal in its topographical relations to the notochord, dorsal aorta and dorsal wall of the alimentary canal. Mesenchymatous cells are plainly proliferated from a well marked area on its median wall, forming an outgrowth comparable to the sclerotome of trunk somites. The somatic wall is plainly epithelial and there is a well marked myocoel. Finally, the somite is, as will be seen later, innervated by a nerve which is generally recognized as comparable with the ventral root of a spinal nerve.

At a 28-30 segment stage this somite is completely separated from its neighbors. Its lumen has become well marked, while anteriorly the somite has assumed a bilobed appearance. At a 32 segment stage this lobation is especially evident. More marked growth now takes place in the middle and posterior part of the somite, and at the same time the walls there begin to lose their epithelial character.

At a 10 mm. stage the lateral posterior end has become distinctly bilobed, and the more lateral of these lobes bears, in favorably orientated sections, a striking resemblance to the visceral portion of the 2nd somite. The disintegration of the epithelial walls of the main portion of the somite continues, while the more dorsal of the two anterior lobes has increased greatly in size. At a 13 mm. stage (Fig. 1) the main

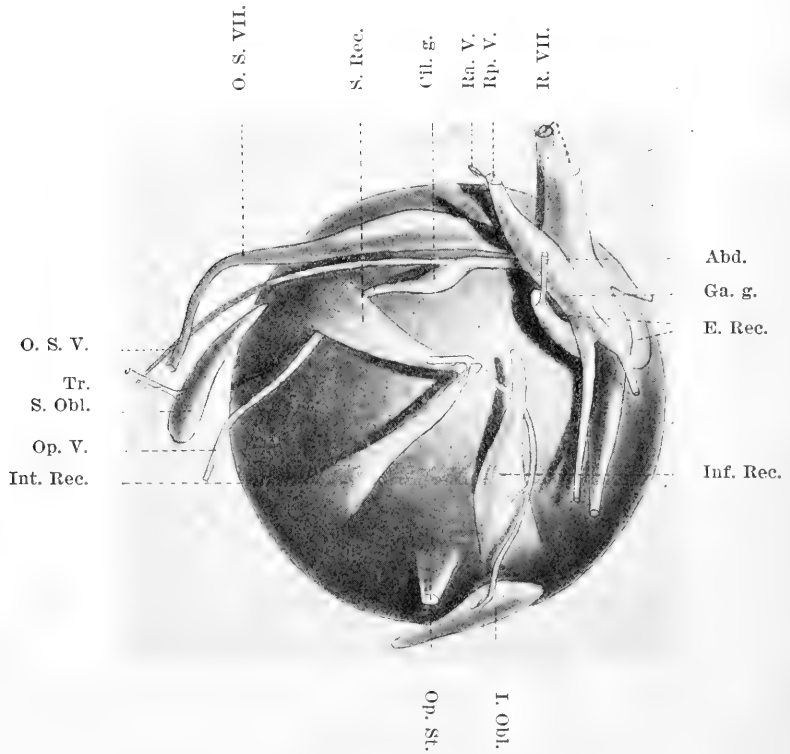

FIG. 8.—Optic vesicle, nerves and eye muscles of an Acanthias embryo, 33 mm. total length. Right side, medial view. The muscles have now nearly their definitive position.

portion of the somite, which is large and globular, is bounded merely by loose connective tissue which now rapidly fills up the lumen of this part of the somite. There is therefore remaining only the anterior prolongation or dorsal lobe, which consists mainly of elements derived from the median wall. This prolongation now grows rapidly and extends directly forward as an elongate pointed process. Its median dorsal wall is thickened, especially at the posterior end. There, as well

as in the anterior portion, cells are assuming an elongate form and a longitudinal direction. The posterior end is indistinct in outline, and cells are evidently dropping off into the mesenchyme. By this degeneration at its posterior end, by growth of the muscle as a whole, and especially by the outpushing at its anterior end (Fig. 9, *E*), the whole somite moves forward, so that while originally located some distance away, it comes to lie in close proximity to the eyeball (Figs. 4, 5, 7, 8, *E. Rec.* Fig. 9, *a*).

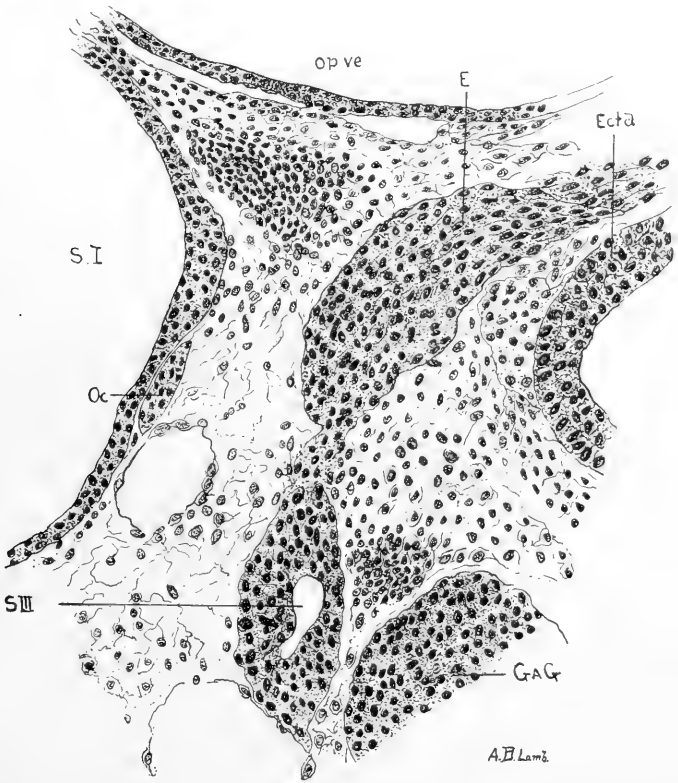

FIG. 9.—Part of transverse section of Acanthias embryo, 19-20 mm. total length, showing the proliferation of the external rectus muscle (E) from the hyoid (III) somite.

This somite is innervated by the abducens. This nerve is, as mentioned above, generally considered as homologous to the ventral motor root of spinal nerves. It arises (Neal, 98, p. 230) at a ten mm. stage from the floor of the hind brain at a point opposite the ear vesicle as an outgrowth from neuroblast cells in the ventral wall of encephalomere VII. The nerve gradually extends forward until it reaches its somite.

Conclusions.

Broadly considered, it will be seen that the necessary mechanical relations between eyeball and muscle is secured: (1) by a forward growth of processes from the 2nd and 3rd somites, and the development of muscle fibres in them; (2) by a spreading out of the 1st somite around the eyeball and the development of muscles in its distal portions.

I wish to call attention to the fact, which so far as I know has not been noticed before, that the original direction of all the eye muscles together with "Muscle E" is longitudinal. This seems to me to represent an originally flexible condition of the head and to be an additional support for the homology of head and trunk somites.

Finally, it seems to me improbable that the present musculature of the eye in Acanthias is the primitive one for several reasons: (1) The adult condition is reached only after the constituent muscles have undergone rather extensive alterations in form and transfer of position. (2) The muscles do not all arise equally early, nor do they reach their definitive condition at the same time. (3) Before some of the permanent eye muscles are formed, one muscle ("Muscle E"), which later disappears, reaches an advanced stage of development. This muscle, from its form and position, must either have once been functionally connected with the eye or with some structure now lost, and of which not even an embryonic rudiment is known. The same reasoning applies to the anterior somite though with diminished force, since it does not reach the same advanced stage of development.

If then the present musculature of the eye is not the primitive one, it becomes an interesting question to inquire if the embryonic development will indicate any stages in the phylogenetic development. Two such stages, it seems to me, are indicated. 1st, a stage where if any eye musculature existed it was furnished by the anterior somite. This is indicated first by the fact that this somite is the only one which from its topographical relations could move the eye; and second, the longitudinal direction and serial arrangement of the remaining muscle *anlagen* indicate a jointed condition of the head and consequently a functional activity on the part of these muscles which would preclude any connection with the eye.

2nd, a stage at which four muscles moved the eye. These were the superior oblique, the external rectus, the inferior oblique and "Muscle E." These four muscles were arranged radially. "Muscle E" and the inferior oblique opposed one another, the former pulling the back of the eye dorsally, the latter, ventrally. The superior oblique and the

external rectus opposed one another, the former pulling the back of the eye forward; the latter, backwards. This stage is reached in ontogeny at a length of 21-22 mm. (Figs. 4, 5, 6). The four muscles then have the rectangular radial arrangement described above. They have all reached approximately the same degree of differentiation, which is far in advance of the three remaining eye muscles.

These speculations, based solely on ontogenetic evidence, require confirmatory phylogenetic evidence derived from a study of forms lower than Acanthias.

REFERENCE LETTERS COMMON TO ALL FIGURES.

Abd., abducens nerve.
AM., *anlage* of muscle adductor mandibulæ.
A. V., auditory vesicle.
BC. I., first branchial cleft.
Cil. g., ciliary ganglion.
C. St., stalk connecting the premandibular somites of the two sides.
E., Temporary muscle derived from the mandibular somite; in Fig. 9, external rectus muscle.
Ect., ectoderm of dorsal surface of head.
E. Rec., externuis rectus muscle.
F., temporary muscle derived from the mandibular somite.
Ga. g., Gasserian ganglion.
I. Obl., inferior oblique muscle.
Inf. Rec., inferior rectus muscle.
Int. Rec., internal rectus muscle.
MS., mesenchyme connecting dorsal and visceral parts of somite II.
NC., notochord.
OC., oculomotor nerve.
Op. St., optic stalk.
Op. Ves., optic vesicle.
Op. V., ophthalmicus profundus branch of fifth nerve.
O. S. V., ophthalmicus superficialis branch of fifth nerve.
O. S. VII., ophthalmicus superficialis branch of seventh nerve.
Ra. V., anterior root of fifth nerve.
Rp. V., posterior root of fifth nerve.
R. VII., root of seventh nerve.
S. A., anterior head somite.
SI., *SII.*, *SIII.*, first (premandibular), second (mandibular), and third (hyoid) somites.
S. Obl., superior oblique muscle.
S. Rec., superior rectus muscle.
SII. D. & V., dorsal and ventral portions of somite II.
Tr., trochlearis nerve.
VII., seventh nerve.

All of the figures except 2 and 9 are drawn from wax reconstructions. The regions ruled on figures 4 and 7 are those portions of the somites which are being transformed into muscles.

BIBLIOGRAPHY.

Dohrn, Anton, '90.—Studien zur Urgeschichte des Wirbelthierkorpers. XV. Neue Grundlagen zur Beurtheilung der Metamerie des Kopfes. Mittheil. Zool. Station, Neapel, Bd. IX, p. 330, 1890.

Hoffmann, C. K., '96.—Beitrage zur Entwicklungsgeschichte der Selachii. Morphol. Jahrbuch, XXIV, pp. 209-286, pls. ii-v, 1896.

Marshall, A. M., '81.—On the head cavities and associated nerves in Elasmobranchs. Quarterly Jour. Micros. Sci., XXI, pp. 72-97, 2 pls., 1881.

Minot, Charles S., '91.—On the morphology of the pineal region, based upon its development in Acanthias. American Journal of Anatomy, i. pp. 81-98, 1901.

Neal, H. V., '98.—The segmentation of the nervous system in Squalus acanthias. Bulletin Mus. Comp. Zool., Harvard College, XXXI, No. 7, pp. 147-294, 9 pls., 1898.

Platt Julia B., '91.—A contribution to the morphology of the vertebrate head based on a study of Acanthias vulgaris. Journal of Morphol., V, pp. 79-112, 3 pls., 1891.

Platt, Julia B., '91a.—Further contribution to the morphology of the vertebrate head. Anatom. Anzeiger, VI, pp. 251-265, 1891.

Van Wijhe, J. W., '82.—Ueber die Mesodermsegmente und die Entwickelung der Nerven des Selachien Kopfes. Natuurk. Verh. Koninkl. Akad. Amsterdam. Deel. XXII, pp. 50, 5 pls., 1882.

Vetter, Benjamin, '74.—Untersuchungen zur vergleichenden Anatomie der Kiemen- und Kiefermusculatur der Fische, Jena. Zeitschrift, VIII, p. 405, 1874.

Zimmermann, K. W., '91.—Ueber die Metamerie des Wirbelthiereskopfs. Verhandl. Anat. Gesellsch. V. (Anat. Anzeiger, VI, Ergänzungs Hefte), pp. 107-114, 1891.

A STATISTICAL STUDY OF THE ABDOMINAL AND BORDER-NERVES IN MAN.

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WITH 8 FIGURES AND 14 TABLES.

The following paper presents the results of a study of the distribution of the main nerves of the abdomen and of the border region between the abdomen and thigh in man. The study was made in the dissecting rooms of the anatomical laboratory of the Johns Hopkins University. The methods employed have been elsewhere described.[1]

Of the ventral branches of the twelve thoracic or intercostal nerves, the first six generally are confined in distribution to the thorax,[2] while the last six are distributed in part to the abdominal walls. In addition, the first two lumbar nerves usually give rise to branches that are distributed to the distal margin of the thoracic wall, and to the skin at the junction of the abdomen and thigh. Considerable variation, however, exists in the origin and distribution of the nerves of the abdomen and of the border region. The extent of this variation in the main nerve tracts is shown in the tables given on pages 216 to 228. The following notes briefly explain these tables:

THE MOST ANTERIOR THORACIC NERVE, THE VENTRAL BRANCH OF WHICH EXTENDS INTO THE ABDOMINAL WALL TO FORM THE "FIRST ABDOMINAL NERVE." *See Table I.*

This table is based upon a very limited number of instances. The ventral branches of the intercostal nerves confined to the thorax emerge ventrally from between two successive costal cartilages. The ventral branches of the nerves distributed both to the abdomen and to the thorax pass below the costal margin of the thorax, then course forwards

[1] (1) A statistical study of the variations in the formation and position of the lumbo-sacral plexus in man, Bardeen and Elting, Anatomischer Anzeiger, 1901, Vol. XIX, p. 124. (2) Use of the material of the dissecting room for scientific purposes, Bardeen, Johns Hopkins Hospital Bulletin, 1901, Vol. XII, p. 155.

[2] With the exception of the fibres distributed by the first two or three to the arm.

between the muscles of the abdominal wall, and finally are distributed in part to the rectus musculature and in part pass through the latter to be distributed to the skin. The last intercostal nerve confined to the thorax is distributed in part to the thoracic segment of the rectus and in part to the overlying skin, while the ventral branches of the more anterior intercostal nerves pass through the overlying structures directly to the subcutaneous tissue. It is probable that occasionally more than one thoracic segment of the rectus muscle is developed in man. Such, however, has not been the case in the subjects which I have examined. As shown in the table, in 10 instances the 6th nerve was the nerve distributed to the thoracic segment of the rectus, and in 6 instances the 7th. The first nerve passing below the costal margin before entering the rectus (that is to say, the first abdominal nerve) was in 10 instances the 7th, and in 6 instances the 8th nerve.

A record was preserved of the race, sex, side of body, skeletal conditions, position of the lumbo-sacral plexus and of the border-nerves in the instances studied. No marked relation seems to exist between any of these factors and the variations noted in the table.

Relations of the Abdominal Nerves to the Transverse Tendons *(Linea transversa, Inscriptiones tendineæ)* of the Rectus Abdominis Muscle. *See Table II.*—The transverse tendons of the rectus abdominis muscle in man correspond to the 7th, 8th, 9th, 10th, and 11th ribs.[3] The relation between the transverse tendons and the costal cartilages is best seen in the transverse tendons corresponding to the seventh and eighth cartilages. In this region the bundles of fibres making up a segment of the rectus originate or terminate in part on the costal cartilage, in part in the corresponding tendon. The transverse tendons corresponding to the eighth costal cartilage are often, to the ninth are usually, and to the tenth and eleventh are always some distance removed from the corresponding costal cartilage.

In the adult individual the transverse tendons are often to a greater or less extent obliterated, owing to the unequal growth of muscle-fibre bundles, some of which extend over more than one segment. The transverse tendon corresponding to the 7th costal cartilage has been fairly distinct in all of the instances I have examined, but that corresponding to the 8th was absent in 9 out of 37 instances (24.3%); to the 9th in 4 out of 62 instances (6.5%); to the 10th in 9 out of 85 instances

[3] See Mall, Devel. of the Ventral Abdominal Walls in Man, Journal of Morphology, Vol. XIV, p. 2, 1898.

(10.6%); and to the 11th in 56 out of 79 instances (70.9%). In no instance have I seen a transverse tendon corresponding to the 12th rib.

In the simple conditions where the segments of the rectus are distinct and the transverse tendons well marked, the abdominal nerves after emerging from the intercostal spaces take a fairly direct course to the lateral margin of the rectus. Each nerve then pierces the rectus sheath, courses along the under surface of the latter muscle, and then gives off one or more cutaneous branches which usually emerge through the rectus in the vicinity of the transverse tendon corresponding to the rib by which the nerve is designated, and one or more muscular branches which are distributed to the segment of the rectus distal to that transverse tendon. The region where the rectus sheath is pierced by a given spinal nerve is usually posterior to the corresponding transverse tendon in case of the 7th and 8th nerves, and anterior in case of the 10th, 11th and 12th nerves. In a recent article in this journal[4] it has been shown that the primary ventral cutaneous branches of the more distal thoracic nerves are caught between the successive tips of those myotomes which give rise to the rectus musculature, and that in this way, the segmental arrangement of the nerves of the abdomen is early insured. In those instances in which no transverse tendon is developed in the region between the tips of two myotomes, the tissue derived from each myotome is fused to a considerable extent, and the corresponding nerves are less definitely guided in their growth. We find, therefore, in the adult far greater irregularity in the course and in the distribution of the branches of such nerves. Often two or more nerve trunks arise from a single intercostal nerve, and course forward to pierce the rectus sheath separately. See Fig. II. This was found to be the case with the 8th nerve, in 2 out of 37 instances; with the 9th in 3 out of 61; with the 10th in 6 out of 85; with the 11th in 18 out of 79 instances; and with the 12th in 17 out of the 56 instances in which the twelfth furnished no direct hypogastric branch. In 18 instances, out of the 74 in which a careful record was made of the branches of the 12th thoracic nerve, the nerve sent a ventral branch to the rectus, and a separate hypogastric branch to the skin of the abdomen. See Fig. III A.

Not infrequently an abdominal nerve will divide into two or more branches immediately before entering the rectus sheath. See Fig. I C.

In the majority of instances, as pointed out by Mall (op. cit.), the transverse tendon of the rectus corresponding to the 10th rib is attached

[4] Bardeen and Lewis: Development of the limbs, body-walls and back in man. This journal, Vol. I, 1901, p. 1.

on its median margin to the dense tissue surrounding the umbilicus. This occurred in 72 out of 85 instances. In 13 out of the 85 instances (15.3%), the transverse tendon corresponding to the 11th rib was intimately united to the tissue surrounding the umbilicus. See Fig. V. In 9 of these instances the transverse tendon corresponding to the 10th rib was absent, in 4, present.

I have discovered no close relation between the development of the 7th, 8th, 9th, 10th, and 11th nerves, and the transverse tendons corresponding to them on the one hand, and race, sex, side of body, skeletal conditions, position of the lumbo-sacral plexus, or distribution of the border-nerves, upon the other.

Origin of the Most Distal Abdominal Nerve Entering the Rectus Muscle. *See Table III.*—As may be seen from the table, the 20th spinal (12th thoracic) nerve is the most distal spinal nerve supplying the rectus muscle in the great majority of instances (96 out of 112 instances, 85.8%).

The nineteenth spinal nerve was the last nerve to furnish fibres to the rectus muscle in but two instances. In both of these the spinal column was shorter than normal, the plexus had an anterior position, and the border-nerves were of a proximal type.[5]

Frequency with which the most distal nerve to the rectus abdominis muscle arose in the types of plexus designated, from the spinal nerves indicated in the column at the left.

Spinal Nerves.	Types of Plexus.													
	A		B		C		D		E		F		G	
	No. of inst.	%	No. of inst.	%	No. of inst.	%	No. of inst.	%	No. of inst.	%	No. of inst.	%	No. of inst.	%
XIX	1	100	1	7.7	6c	..	..	..	..	..	..	..	..	..
XX	..	..	12	92.3	33	100	30	90%	14	78.8	9	90	5	55.5
XXI	..	..	..	..	..	..	3	10%	4	22.2	1	10	4	44.5

[5] Owing to an unfortunate oversight, a number of the earlier charts, made at a time when especial care was not taken in the study of the abdominal nerves, were included in making up the column on the "last nerve to the rectus muscle" in the Tables 2-8, in the article by Bardeen and Elting, on "A statistical study of the variations in the formation and position of the lumbo-sacral plexus in man." Anatomischer Anzeiger, Vol. XIX, pp. 228-237, 1901. These earlier charts should have been excluded in making up this column. The following table is based upon charts which record with especial exactness the more distal nerves of the abdomen:

Of the 14 instances in which the 21st nerve furnished fibres to the rectus muscle, in 9 the vertebral column was apparently normal, and in 5 it was lengthened by an additional vertebra. In 2 instances the plexus was of the normal type, in 10, of the distal type, and in 2 instances no good record of the plexus was preserved. See Fig. V.

No marked relations were noted between these variations in the distal supply of the rectus muscle and sex, race or side of body. Both Ruge [6] and Bolk [7] have given interesting accounts of the relations of the distal abdominal and border-nerves in the anthropoid apes.

The Number of Spinal Nerves Contributing to the Nerve Supply of the Abdomen. *See Table IV.*—In connection with the abdominal nerves it is of interest to inquire how many spinal nerves contribute to the nerve supply of the abdomen. With the possible exception of twigs furnished by the most distal nerve confined to the thorax to the most anterior portion of the transversalis abdominis muscle, the first nerve of supply of the abdomen is the most anterior intercostal nerve the ventral branch of which passes below the costal margin to enter the abdominal wall. Similarly, with the exception of twigs furnished now and then by the genito-crural nerves, the inguinal nerve is the most posterior nerve furnishing a nerve supply to the abdominal walls. Taking the 1st abdominal nerve as the anterior limit, and the inguinal nerve as the posterior limit, we find that in 10 out of 16 instances (62.5%), seven spinal nerves contributed to the supply of the abdominal wall, in 4 instances (25%) six nerves, and in 2 instances (12.5%) five nerves thus contributed.

The transversalis and internal oblique muscles are supplied by branches which spring from the main abdominal nerves during their course to the rectus, and from the ileo-hypogastric, ileo-inguinal, and sometimes from the genital nerve, during their course between these muscles. The last nerve also furnishes fibres to the cremaster muscle. The muscular branches springing from these various nerves are irregular in origin and distribution, and give rise to a plexiform union between successive nerve trunks. Owing to the great irregularity of these secondary muscle branches no statistical data concerning them are furnished.

[6] T. Ruge: Verschiebungen in den Endgebieten der Nerven des Plexus lumbalis der Primaten. Morph. Jahrbuch X, 1893, p. 305.

[7] Beitrag zur Neurologie der unteren Extremität der Primaten. Morph. Jahrbuch XXV, 1898, p. 305.

The lateral branches of the intercostal nerves furnish nerves to the external oblique muscle and cutaneous branches which vary much in distribution. I have seen, for instance, a combined nerve trunk, arising from the lateral branches of the 11th and 12th thoracic nerves, extend well into the pubic region. The charts furnish, however, insufficient data on which to base a statistical study of variations in the lateral cutaneous branches of the eleven more anterior intercostal nerves. On page 210 the relation of the lateral branch of the 12th intercostal nerve to the iliac region is considered.

The Various Types of Distribution of the Border-Nerves. *See Table V, and Figs. 1-8.*—Greater variation seems to exist in the origin of the border-nerves from spinal nerves than in the origin of the main abdominal nerves. With the exception of the genito-crural nerves, however, the courses taken by the border-nerves are fairly definite and are well described in the standard text-books. We shall consider first the variation in origin of the border-nerves taken as a group, and then that of the individual border-nerves.

In Table V, we have divided the sets of border-nerves found in the subjects studied into various types. The five main types are based upon the spinal nerves from which the border-nerves arise. In Type I, all border-nerves arise from the 20th and 21st spinal nerves; in Type II, from the 21st; in Type III, from the 20th, 21st and 22nd; in Type IV, from the 21st and 22nd; and in Type V, from the (20), 21st, 22nd and 23rd. Types I, III and IV are further subdivided into sub-types, according to the relation of the spinal nerves to the individual border-nerves. These relations are made clear by the table. It will be noted that the border-nerves most frequently arise from the 20th, 21st and 22nd spinal nerves, and next most frequently from the 21st and 22nd. Types III and IV A., the forms most commonly met with, correspond with the pictures given in most text-books to illustrate the normal type.

Relations of Race, Sex and Side of Body to the Various Types of Distribution of the Border-Nerves. *See Table VI.*—In Table VI are given the relation of race, sex and side of body to the various types of distribution of the border-nerves. It will be noted that no very marked relations of this nature seem to exist. A much larger number of instances than we have studied would be necessary before reliable deductions could be drawn as to the influence of these factors in determining variation in the distribution of the peripheral nerves under discussion.

Relative Distribution of the Border-Nerves on Each Side of the Body. *See Table VII.*—There is considerable variation in the types of distribution exhibited by similar nerves on the two sides of the same body. In Table VII the relation of the types of distribution of the border-nerves on one side of the individual to those on the other are given. Each numeral in the body of the table indicates the number of instances in which the type of distribution indicated at the left of the table was found associated with the type of distribution indicated at the top of the column. Thus, in two instances, when Type I D. was found on the right side, Type I C. was found on the left. The heavy figures indicate that the type of distribution of the border-nerves was the same on each side of the body in the number of individuals denoted by the figure. Thus, Type I B. was found on each side of the body in two individuals. It will be noted that while slight variations in type of distribution is very common, marked variation is rare.

Relation of Variations in the Spinal Column to the Various Types of Distribution of the Border-Nerves. *See Table VIII.*—This table shows that a close relation exists between the development of the spinal axis and the distribution of the border-nerves. Reduction in the spinal axis is marked in extreme cases by the loss of a thoracic, lumbar or sacral vertebra. In less extreme instances there is a tendency for the twelfth thoracic vertebra to assume the lumbar type, for the fifth lumbar to assume the sacral type, and for the fifth sacral to assume the coccygeal type. Although occasionally the twelfth rib may be ill-developed without accompanying changes in the lumbar and sacral vertebra, as a rule a rudimentary 12th rib indicates a tendency to a shortening of the spinal axis, as outlined above. When the spinal axis is reduced, the hip bones are attached to the spinal axis more anteriorly than is usual, although not necessarily to the 24th vertebra. This anterior position of the posterior limb accounts for the derivation of the border-nerves from a more anterior set of spinal nerves than normal, Types I A., B., C., D., E., II and III A. and B. On the other hand, types of border-nerves marked by a more distal origin than normal (IV B. & V.), are characterized by the great frequency with which they are associated with extension in the vertebral column, marked in extreme cases by the addition of an extra thoracic, lumbar, or sacral vertebra.

Types of Lumbo-Sacral Plexus Associated with the Various Types of Distribution of the Border-Nerves. *See Table IX.*—As

might be expected from the intimate relations existing between the development of the spinal axis, the position of the posterior limb, and the type of distribution of the border-nerves, we find also that when the lumbo-sacral plexus has a more anterior position than usual, the border-nerves generally arise from an anterior set of spinal nerves; and that when the lumbo-sacral plexus has a posterior position, the border-nerves likewise arise from a distal set of spinal nerves.[8]

Origin of the Hypogastric Nerve. *See Table X.*—We shall now consider in turn the individual border-nerves, beginning with the hypogastric.

In 6 instances, 2% of the number studied, the hypogastric nerve arose from the 19th and 20th spinal nerves; in 91 instances, 32%, from the 20th; in 98, 34%, from the 20th and 21st; and in 92, 32%, from the 21st. In 106 instances, 37%, the hypogastric nerve arose from the ventral trunk of the 20th spinal nerve; in 190 instances, 69%, from the 21st; in 9 instances, 3%, there were two hypogastric nerves.

In the table the term "dorsal origin" is used to indicate the separation of the hypogastric nerve from the main ventral trunk of the twelfth thoracic nerve near the spinal axis. See Fig. 1, A. The term "ventral origin" is used to indicate the separation of the hypogastric nerve from the main ventral trunk after the latter has extended well into the abdominal wall. See Fig. 1, C. The hypogastric has a dorsal origin from the twelfth nerve in 40 instances, and a ventral origin in 43 instances out of the 83 in which these conditions were tabulated distinctly.

Origin of the Iliac Nerves. *See Table XI.*—By the term "iliac nerve" is meant a nerve which passes over the crest of the ilium to be distributed on the lateral surface of the hip. Only those nerves have been called "iliac nerves" the distribution of which is entirely distal to the level of the iliac crest. In addition, the lateral branches of the more distal intercostal nerves often extend in their distribution from the region of the abdomen over the lateral region of the hip.

In 3 instances, 1%, distinct iliac nerves were given off from the 19th spinal (11th thoracic) nerve; in 110 instances, 40%, iliac nerves were derived directly from the 20th spinal nerve; and in 76 additional instances, 27%, from the 21st, after the latter had received a branch of

[8] The relation between the lumbo-sacral plexus and the development of the spinal-axis has been pointed out elsewhere.—Bardeen and Elting, op. cit. p. 203.

communication from the 20th. The iliac nerve was derived from the 21st spinal nerve in 198 instances, 70.4%. The mode of origin of the iliac nerve is indicated in the table. Most commonly (221 instances, 86%) it arises as a branch from the hypogastric nerve as this passes near the iliac crest. In 43 instances, 15.3%, it arose as a branch of the main ventral trunk of the 20th spinal nerve. Less frequently (21 instances, 7.5%) it passed as a separate trunk from the region of the spinal axis (dorsal origin) to the crest of the ilium. In only 23 instances, 8.2%, was an iliac nerve found to arise from the inguinal. The term ilio-inguinal should be restricted to nerves of this character, which are comparatively rare. Two iliac branches are not infrequent.

Origin of the Inguinal Nerve.—The inguinal nerve in the great majority of instances arises from the 21st spinal nerve (258 instances, 89.8%). In nearly half of these instances (110) fibres were also derived through a proximal communicating branch from the 20th spinal nerve. In 10 instances, 3.5%, it arose from the 20th spinal nerve, and in 19 instances, 6.6%, the place of the inguinal nerve was taken by the genital branch of the genito-crural. Most commonly (224 instances, 78%) the inguinal nerve takes a course to the iliac crest separate from that of the hypogastric. Not infrequently (36 instances, 12.5%), however, these two nerves pass in a common trunk as far as the iliac crest, and infrequently (8 instances, 2.8%) they pass in a common trunk to the region of the external ring, whence the hypogastric branch turns up over the abdomen, while the inguinal nerve takes its way to the region where scrotum and leg adjoin.

Origin and Course of the Genito-crural Nerves. *See Table XIII.*—So great is the variety in the distribution of the genito-crural nerves, it would be necessary to describe nearly every subject examined in order to record the many different courses taken by these nerves. The main trunks of the abdominal nerves are kept fairly constant in distribution, owing to their relation to the rectus muscle. The hypogastric and inguinal nerves are kept within moderate bounds, owing to their course in channels between the transversalis and internal oblique muscles, and between the latter and the flat tendon of the external oblique, channels that are limited distally by the crest of the ilium and by Poupart's ligament. Not infrequently the inguinal nerve courses for some distance between the abdominal fascia and the transversalis muscle before piercing the latter and entering the channel offered between it and the internal oblique muscle. In the region of its attach-

ment to the ventral portion of the iliac crest the internal oblique muscle is often divided into two layers, and between the layers another channel is offered for the passage of the inguinal and hypogastric branches. But while the regions in which the hypogastric and inguinal nerves pass from the channel between the transversalis fascia and the transversalis muscle to that between the latter and the internal oblique muscles, and from this to the channel between the two layers of the internal oblique and thence to that between the internal oblique and the tendon of the external oblique, vary in different individuals, the general course of these nerves is fairly constant. On the other hand, there are no definite paths of guidance for the genito-crural nerves. They take an irregular course from the anterior region of the lumbo-sacral plexus through the psoas muscle and behind the transversalis fascia to the region of Poupart's ligament. Here the genital nerve pierces the transversalis and internal oblique muscles or their tendon enters the channel between the latter and the tendon of the external oblique, fuses here with the inguinal nerve, and is distributed in common with the branches of the latter nerve. The crural nerve, on the other hand, passes below Poupart's ligament and supplies the skin of the leg near the region of its junction with the abdomen. Either or both nerves may give off branches to the external iliac and femoral arteries.

In origin, the genito-crural nerves vary no more than the other border-nerves. Thus, from Table V it will be seen that the genito-crural nerves arise from the 21st nerve in 56 instances, 19% (in a certain number of these some fibres are derived from the 20th also); from the 21st and 22nd in 125 instances, 79%; and in but 6 instances, 2%, from the (21st), 22nd and 23rd.

There is considerable variation in the number of nerves designated "genito-crural." Most commonly (in 154 instances out of 250 of which good records are preserved, 61.6%), the genital and crural branches are bound in a common trunk, which, at a variable distance above Poupart's ligament, divides into genital and crural branches. Not infrequently, in addition to such a trunk, there is an extra genital branch (16 instances, 6.4%), or an extra crural branch (25 instances, 10%). Occasionally no crural branch is found (3 instances, 1.2%); more often the genital branch is wanting (17 instances, 6.8%). I have seen no instances in which both branches were wanting.

The regions of exit of the genital nerve into the path taken by the inguinal nerve and of the crural nerve into the superficial fascia of the thigh, vary greatly. The genital nerve may pass into the path of the inguinal not far from the anterior superior spine of the ilium (lateral

region of emergence, 47 out of 121 instances, 38.9%, see Fig. V), or in the vicinity of the femoral nerve (middle region of emergence, 55 instances, 45.5%, see Fig. II), or near the pubic crest (median region, 19 instances, 15.6%, see Fig. IV. A.). The crural nerve may pass to the leg in corresponding regions (lateral emergence, 27 out of 133 instances, 20.3%, see Fig. I. C.), middle emergence, 81 instances, 60.9%, see Fig. I. A, median emergence, 25 instances, 18.8%, see Fig. III. E.).

When the genital nerve passes into the path of the inguinal near the anterior superior spine, it assumes many of the characteristics of the inguinal nerve. The inguinal nerve may take a course to the extreme ventral limit of the iliac crest before passing into the abdominal musculature. Between an inguinal nerve of this type and a genital nerve emerging in a lateral region, only an artificial distinction can be drawn. As the line of demarkation between the two, I have taken the anterior superior spine.

The crural nerve, when it emerges laterally, shows a tendency to become more or less closely united to the lateral cutaneous nerve of the thigh. Occasionally the crural nerve arises as a branch of the lateral cutaneous (9 instances out of 287, 3.1%). In one instance only have I seen a genital nerve arising as a branch of the lateral cutaneous nerve of the thigh.

Origin of the Lateral Cutaneous Nerve of the Thigh. *See Table XIV.*—In connection with the border-nerves it may be of interest to consider briefly the origin of the lateral cutaneous nerve in connection with the various types of distribution of the border-nerves. In the more "anterior" types of border-nerves, it will be noted that the lateral cutaneous nerve springs most frequently from the 21st and 22nd spinal nerves, while in more posterior types it springs from the (21st), 22nd and 23rd, or from the main trunk of the femoral nerve. This association is not, however, a constant one.

General Conclusions.

Variation in the abdominal and border-nerves may be due either to local conditions, which affect merely the nerves derived from a given spinal segment and their immediate neighbors, or it may be due to conditions which affect the whole distal region of the spinal axis, and the position of the limb relative to the axis. Race, sex and side of body seem to have no specific influence in determining variation of either sort.

Variation in the abdominal nerves anterior to the twelfth intercostal

seems, in the main at least, to be due to local conditions. Not infrequently the nerves and musculature derived from a given spinal segment have a less extensive development than usual. In such instances, the musculature derived from this segment gives way in part to musculature derived from a neighboring segment, and the nerve belonging to the latter covers a territory nearly equal to twice the usual territory, while the nerve belonging to the less developed segment is much restricted in distribution. These conditions become clear in a study of the rectus muscle. Similar variations in extent of the cutaneous territory covered by a given spinal nerve, are also frequent.

The border-nerves may exhibit individual variations, or they may be affected as a group. In the latter case, the variations are intimately associated with the length of the spinal axis and the position of the posterior limb. This association is shown in Table VIII. When the border-nerves spring from the 20th and 21st spinal nerves merely, the condition is, with very few exceptions, found associated with skeletal conditions, which indicate a reduction in the vertebral axis. In the three exceptions given under Type I, we may assume that the genito-crural nerves were locally affected, and that the condition in these three instances does not indicate an influence exhibited on the border-nerves as a whole. The association of the more distal types of distribution of the border-nerves with extension in the vertebral column is less marked, owing probably to the fact that the relation of the pelvic bones to the vertebræ and the form of the vertebræ were recorded only in the more extreme instances of extension to the amount of a full segment. The frequency and the extent of the variation in the spinal origin of the border-nerves and of the lumbo-sacral plexus, make it important that these factors should be taken into account in making up tables of nerve distribution, like the valuable tables of Head. The marked relation existing between these variations and variations in skeletal conditions is their most noteworthy feature.

In the valuable paper by Bolk, referred to above (p. 207), he points out that the lumbo-sacral plexus in the anthropoid apes is as a rule in an anterior, or "high," position as compared to man, and that distinct border-nerves are less well developed. In man, however, when the spinal axis is shorter than usual and the lumbo-sacral plexus has an anterior position, we do not, as a rule, find that the border-nerves are reduced in number, although they may arise from but a single spinal nerve. Peripheral courses for nerve development are developed somewhat independently of the relation of the position of the limb to the spinal axis.

In an interesting communication by P. Ancel and L. Sencert,[1] these authors take exception to the terms "anterior" and "posterior" or "pre-fixed" and "post-fixed" as applied to the various types of variation in origin found in the lumbo-sacral plexus and the neighboring nerves. It must be admitted that local variation in the place and extent of origin of the border-nerves, and the nerves of the limb is more frequent than is marked variation in the relation of all of these nerves as a group to the segmental spinal nerves. The correlation, however, often found between variation in origin of the border-nerves and the position of the lumbo-sacral plexus on the one hand and the development of the spinal axis upon the other, makes it seem well to retain the terms "anterior" and "posterior" in describing variation in origin of these nerves.

[1] Contribution a l'étude du plexus lumbaire chez l'homme, Bibliographie anatomique IX, 1901, p. 209.

TABLE I.

The Relations of the Ventral Branches of the Sixth, Seventh and Eighth Intercostal Nerves to the Rectus Muscle, Thorax and Abdomen.

Most anterior attachment of rectus is at	No. of instances.	1st intercostal nerve entering abdominal wall.		Nerve entering rectus from between two costal cartilages.	
		7th. No. of instances.	8th. No. of instances.	6th. No. of instances.	7th. No. of instances.
4th costal cartilage	1	1	..	1	..
5th " "	7	7	..	7	..
6th " "	8	2	4	2	4
7th " "	2	..	2	...	2
Totals	18	10	6	10	6

TABLE II.

The Frequency of the Presence and Absence of Transverse Tendons (*Linea transversa, Inscriptiones tendineæ*) Corresponding to the 8th, 9th, 10th and 11th Ribs, and the Relations of the Tenth and Eleventh Tendons to the Umbilicus.

	Tendon Corresponding to the			
	8th rib.	9th rib.	10th rib.	11th rib.
Present:				
No. of Instances	*28*	*58*	*76*	*23*
Per cent	75.7	93.5	89.4	29.1
Absent:				
No. of Instances	*9*	*4*	*9*	*56*
Per cent	24.3	6.5	10.6	70.9
Median Margin near Umbilicus:				
No. of Instances	..	..	*72*	*13*
Per cent	..	..	84.7	15.3

TABLE III.

ORIGIN OF THE MOST DISTAL ABDOMINAL NERVE ENTERING THE RECTUS MUSCLE.

Nerve arose from the XIX spinal nerve in 2 instances	1.8%
Nerve arose from the XX spinal nerve and from a communicating branch from the XIX in 7 instances	6.3
Nerve arose solely from the XX spinal nerve in 89 instances	79.5
Nerve arose from the XX and XXI spinal nerves through anastomosis in 5 instances	4.5
Nerve arose solely from the XXI spinal nerve in 9 instances	8.0

TABLE IV.

NUMBER OF SPINAL NERVES DISTRIBUTING BRANCHES TO THE ABDOMINAL MUSCLES.

7 nerves contribute:		
1st abd. nerve from XV spinal, last nerve to rectus from XX spinal, inguinal from XXI spinal	10	instances
6 nerves contribute:		
1st abdominal from XVI spinal, last nerve to rectus from XX spinal, inguinal from XXI spinal	4	"
5 nerves contribute:		
1st abd. nerve from XVI spinal, last nerve to rectus from XX spinal, inguinal from XX spinal	2	"

TABLE V.

The Various Types of Distribution of the Border-nerves.

(Total number of instances studied, 287. See Figures on opposite page.

I. *The border-nerves arise from the 20th and 21st spinal nerves.* 50 instances, 17% of the total number.
   - A. Hypogastric and inguinal nerves arise from the 20th spinal nerve, the genito-crural from the 21st, after this has received a communicating branch from the 20th. 5 instances, 10%. See Fig. I A.
   - B. Like A, but with no communicating branch, 6 instances, 12%.
   - C. The hypogastric nerve arises from the 20th spinal, the inguinal and genito-crural nerves arise from the 21st, after this has received a communicating branch from the 20th. 21 instances, 42%. See Fig. I C.
   - D. Like C, but with no communicating branch. 11 instances, 22%.
   - E. The hypogastric nerve arises from the 21st spinal, after this has received a communicating branch from the 20th. The inguinal and genito-crural nerves likewise arise from the 21st. 7 instances, 14%.

II. *All border-nerves arise from the 21st spinal nerve.* 6 *instances, 2% of total number.* See Fig. II.

III. *The border-nerves arise from the 20th, 21st, and 22nd spinal nerves.* 139 *instances, 49% of total number.*
   - A. The hypogastric nerve arises from the 20th spinal, the inguinal from the 21st and from a proximal communicating branch from the 20th, the genito-crural from the 21st and 22nd. 27 instances, 19%. See Fig. III A.
   - B. Like A, but with no proximal communicating branch. 27 instances, 19%.
   - C. The hypogastric and inguinal nerves arise from the 21st spinal nerve and from a proximal communicating branch from the 20th, the genito-crural arises from the 21st and 22nd spinal nerves. 68 instances, 49%. See Fig. III C.
   - D. Like C, except that none of the fibres from the 20th spinal nerve go into the inguinal nerve. 8 instances, 6%.
   - E. Two hypogastric branches, one from the 20th and one from the 21st spinal nerves, inguinal from 21st, genito-crural from 21st and 22nd. 9 instances, 7%. See Fig. III E.

IV. *The border-nerves arise from the 21st and 22nd spinal nerves.* 86 *instances, 30% of total number.*
   - A. Hypogastric and inguinal nerves from the 21st, genito-crural from the 21st and 22nd. 78 instances, 91%.
   - B. Hypogastric and inguinal nerves from the 21st, genito-crural from the 22nd. 8 instances, 9%.

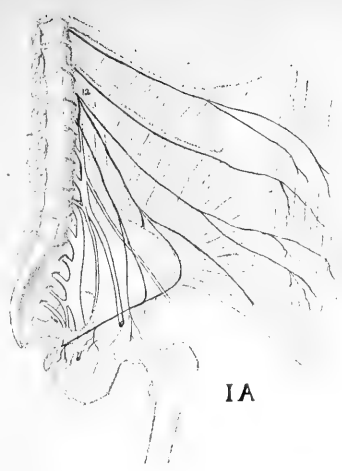
V. *The border-nerves arise from the 21st, 22nd and 23rd spinal nerves.* Hypogastric from the 20th and 21st spinal nerves, inguinal from the 21st and 22nd, genito-crural from the 22nd–23rd. 6 instances, 2% of total number.

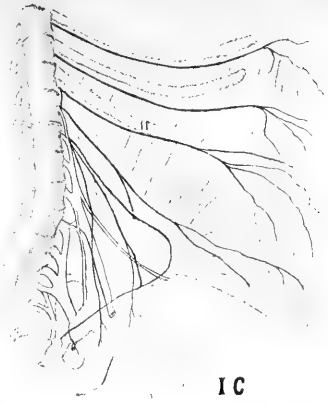
DESCRIPTION OF THE FIGURES ON OPPOSITE PAGE.

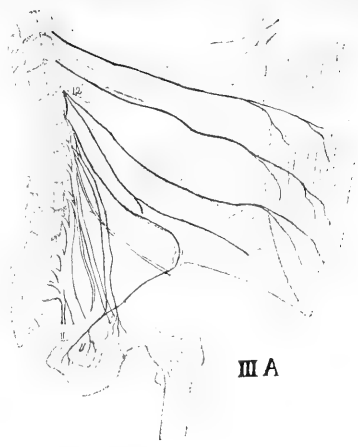
These figures represent the distal abdominal and the border-nerves of various types in their relation to the abdominal wall, spinal column, skeleton of the limb and lumbo-sacral plexus. The ventral portion of the abdominal wall is shown turned back. The transversalis muscle is not represented. In the rectus muscle the transverse tendon corresponding to the tenth rib is shown in all figures except Fig. V. A transverse tendon corresponding to the eleventh rib is shown in Figures IV A and V. In each figure the hypogastric nerve is represented passing through the internal oblique muscle near its distal margin and at a point about half the distance between the anterior superior spine of the ilium and the distal extremity of the rectus. The inguinal nerve is shown coursing from the anterior superior spine of the ilium to the crest of the pubis. The genital and crural nerves pass from the pelvis in various regions, the genital branches in each instance becoming united to the inguinal nerve while the crural branches pass out to the region of the leg. The nerves of the limb arising from the lumbo-sacral plexus are represented diagrammatically in double outline. The lateral cutaneous nerve passes to a point near the anterior superior spine of the ilium, the femoral nerve passes to a point over the head of the femur, the obturator emerges through the obturator foramen, the sciatic nerve passes behind the ischium and the pudic nerve passes between the great and lesser ischio-sacral ligaments.

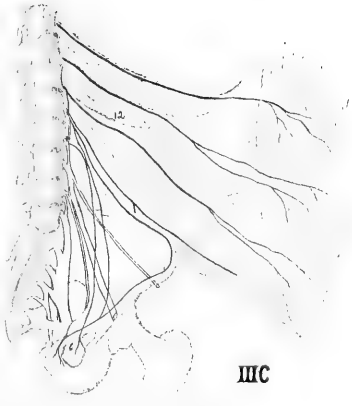
The twelfth rib is denoted by the appropriate numeral, except in Fig. I C., where the eleventh is thus designated.

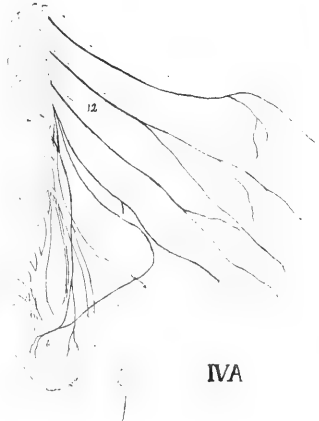
TYPES OF DISTRIBUTION OF THE BORDER-NERVES.











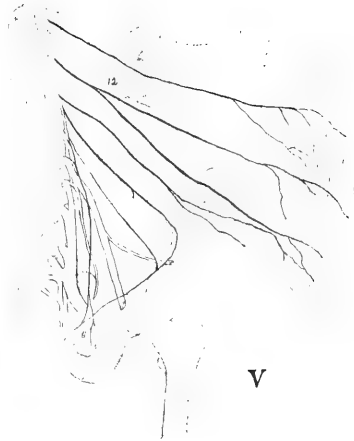


TABLE VI.

RELATIONS OF RACE, SEX, AND SIDE OF BODY TO THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

			Totals.		Type I.							Type II.		Type III.							Type IV.				Type V.	
					A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
			No. of instances.	%	No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
Negro,	Male.....	R.	*63*	22	1	2	4	2	4	*13*	26	*1*	17	4	5	21	..	1	*31*	23	15	2	*17*	20	*1*	17
		L.	*63*	22	2	2	3	1	1	*9*	18	*1*	17	6	4	16	3	5	*34*	25	15	2	*17*	20	*2*	33
	Female...	R.	*38*	13	..	1	3	3	..	*7*	14	*2*	33	3	2	7	1	..	*13*	9	14	..	*14*	16	*2*	33
		L.	*34*	12	..	..	3	..	1	*4*	8	*1*	17	5	2	8	1	1	*17*	12	9	2	*11*	13	*1*	17
White,	Male.....	R.	*41*	15	..	1	4	2	..	*7*	14	*1*	17	3	5	9	2	1	*20*	15	12	1	*13*	15	..	..
		L.	*35*	12	..	..	3	3	1	*7*	14	..	..	3	5	7	1	..	*16*	12	11	1	*12*	14	.	..
	Female...	R.	*5*	2	1	..	1	..	..	*2*	4	..	..	..	2	..	..	..	*2*	2	1	..	*1*	1	..	..
		L.	*6*	2	1	..	..	..	..	*1*	2	..	..	1	2	..	..	1	*4*	3	1	..	*1*	1	..	..
Totals.....			*285*		5	6	21	11	7	*50*		6		25	27	68	8	9	*137*		78	8	*86*		*6*	

TABLE VII.

THE RELATIVE DISTRIBUTION OF THE BORDER-NERVES ON EACH SIDE OF THE BODY.

Number of Instances in which the Various Types of Border-nerves were found on Right Side.				Number of Instances in which the Various Types of Border-nerves were Found on Left Side.														
				I					II	III					IV		V	
				A	B	C	D	E		A	B	C	D	E	A	B		
				3	2	10	4	3	2	16	13	28	5	7	35	5	3	
I	A	2		**2**	..	..	..	..	..	.	..	..	..	..	..	..	..	
	B	4		..	**2**	1	..	..	..	..	1	..	..	..	..	..	..	
	C	11		1	..	**4**	..	1	..	4	1	..	..	..	..	..	..	
	D	7		..	..	2	**1**	..	..	..	3	..	1	..	..	..	..	
	E	3		..	..	..	..	..	..	..	..	1	..	1	1	..	..	
II		4		..	..	..	1	..	..	1	1	..	1	..	..	..	..	
III	A	11		..	..	1	1	1	1	**4**	..	..	2	..	1	..	..	
	B	13		..	..	..	..	..	1	4	**6**	1	..	..	..	1	..	
	C	32		..	..	2	..	1	..	1	..	**11**	1	2	14	..	..	
	D	3		..	..	..	..	..	..	..	..	2	..	..	..	1	..	
	E	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	
IV	A	40		..	..	..	1	..	..	1	1	12	..	4	**17**	2	2	
	B	3		..	..	..	..	..	..	..	..	..	..	..	2	**1**	..	
V		3		..	..	..	..	..	..	1	..	1	..	..	..	..	*1*	

TABLE VIII.

RELATION OF VARIATIONS IN THE VERTEBRAL COLUMN TO THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Condition of vertebral column	Totals. No.	Totals. Per cent.	Type I. A No.	B No.	C No.	D No.	E No.	Type I. Total No.	Type I. Per cent.	Type II. Total No.	Type II. Per cent.	Type III. A No.	B No.	C No.	D No.	E No.	Type III. Total No.	Type III. Per cent.	Type IV. A No.	B No.	Type IV. Total No.	Type IV. Per cent.	Type V. Total No.	Type V. Per cent.
Reduction in vertebral column	..	..	..	..	..	..	..	..	..	..	..	.	..	..	.	..	..	..	..	.	..	..	..	..
Vertebræ 11 t. 5 l. 5 s. 3 c.....	*4*	9	2	..	1	1	..	*4*	21	..	..	..	.	..	..	..	..	..	..	..	..	..	..	..
11 t. 5 l. 6 s. 2 c.....	*2*	4	..	.	..	1	..	*1*	5	..	..	..	1	..	..	..	*1*	5	..	..	..	..	..	..
12 t. 4 l. 5 s. 3 c.....	*1*	2	..	..	1	..	..	*1*	5	..	..	..	..	..	..	.	..	..	..	..	..	..	..	..
12 t. 5 l. 4 s. 3 c.....	*2*	4	..	..	..	..	..	..	..	*1*	33	1	..	..	..	..	*1*	5	..	..	..	..	..	..
12th rib rudimentary	*37*	80	2	1	3	6	1	*13*	69	*2*	67	5	6	4	.	3	*18*	90	4	..	*4*	100	..	..
Total No. assoc. with reduced spinal axis..	**46**	41	4	1	5	8	1	**19**	86	**3**	75	6	7	4	..	3	**20**	40	4	..	**4**	12	..	..
Normal vertebral column.....	**54**	48	..	..	1	..	2	**3**	14	**1**	25	1	2	19	2	3	**27**	54	21	2	**23**	65	..	..
Extension in vertebral column	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Vertebræ 12 t. 5 l. 6 s. 4 c.....	*2*	17	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	*2*	25	..	..
12 t. 6 l. 5 s. 4 c.....	*4*	33	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	1	*4*	50	..	..
12 t. 6 l. 4 s. 3 c.....	*2*	17	..	..	..	..	..	..	..	..	..	..	..	1	..	..	*1*	33⅓	1	..	*1*	13	..	..
13 t. 5 l. 5 s. 4 c.....	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
13 t. 5 l. 4 s. 3 c.....	*2*	17	..	.	..	..	..	..	..	.	..	..	..	1	..	..	*1*	33⅓	..	..	..	..	**1**	100
13 t. 4 l. 6 s. 3 c.....	*2*	17	..	..	..	..	..	.	..	..	..	..	..	1	..	..	*1*	33⅓	1	..	*1*	13	..	.
Total No. assoc. with extended spinal axis.	**12**	11		..	..	..	..	..	..	..	..	..	..	3	..	..	**3**	6	6	2	**8**	23	**1**	..

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TABLE IX.

TYPES OF LUMBO-SACRAL PLEXUS ASSOCIATED WITH THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Position of the lumbo-sacral plexus.				Totals		Frequency with which a given form of plexus was found associated with a given type of distribution of the border-nerves.																					
						Type I.							Type II.		Type III.							Type IV.				Type V.	
Type of plexus.	Most anterior spinal nerve to supply nerves of posterior limb.	Furcal nerve.	Most posterior spinal nerve to supply nerves of posterior limbs.	No. of instances.	Per cent.	A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
						No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
A	XXI	XXIV	XXVI	*1*	1	..	..	..	..	..	..	..	..	..	1	..	..	..	..	*1*	1	..	..	..	..	..	..
B	XXI or XXII	(XXII), XXIV	XXVII	*25*	10	1	2	7	2	1	*13*	29	*2*	40	1	3	2	1	..	*7*	6	3	..	*3*	4		..
C[1]	XX, XXI, or XXII	(XXIII), XXIV	XXVIII	*63*	26	4	3	10	8	3	*28*	62	*1*	20	9	8	7	..	3	*27*	24	7	..	*7*	9	..	..
D[1]	XXI or XXII	XXIV	XXVIII	*104*	43	..	..	2	..	2	*4*	9	*2*	40	6	10	34	5	4	*59*	51	37	2	*39*	53	..	.
E	XXI or XXII	XXIV-XXV or XXV	XXVIII	*17*	7	..	..	..	..	..	..	..	..	.	2	..	6	1	..	*9*	8	6	1	*7*	9	*1*	33
F	XXI or XXII	XXIV	XXIX	*15*	6	..	..	..	..	..	..	..	.	..	..	1	3	..	1	*5*	4	8	2	*10*	13	..	..
G	XXI or XXII	XXV	XXIX	*18*	7	..	..	..	..	..	..	..	..	..	..	..	7	..	..	*7*	6	8	1	*9*	12	*2*	67
			Total..	*243*	..	5	5	19	10	6	*45*	..	*5*	..	19	22	59	7	8	*115*	..	69	6	*75*	..	*3*	..

[1] In C the greater portion of the furcal nerve goes into the posterior nerves of the limb; in D, into the anterior nerves of the limb.

TABLE X.

ORIGIN OF THE HYPOGASTRIC NERVE IN THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Hypogastric nerve arises	Totals.		Type I.							Type II.		Type III.							Type IV.				Type V.	
			A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
	No.	%	No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
From XIX and XX spinal nerves (fibres from XIX are derived through prox. com. branch)	**6**	2	1	..	3	..	..	**4**	8	..	..	2	..	..	.	..	**2**	1.4	..	.	..	..	..	..
Dorsal origin.....	2	..	.	..	2	..	..	*2*	..	..	..	.	..	..	..	..	..	..	.		..	..	..	..
Ventral origin.....	3	..	1	..	1	..	..	*2*	..	..	..	1	.	..	..	..	1	..	..	..	..	..	..	..
From XX spinal nerve....	**91**	32	4	6	18	11	..	**39**	78	..	..	25	27	..	..	..	**52**	37.5	..	..	..	..	..	..
Dorsal origin.....	38	.	2	4	4	7	..	*17*	..	..	..	12	9	..	..	..	*21*	..	..	..	..	..	.	..
Ventral origin.....	31	..	2	..	6	3	..	*11*	.	..	..	9	11	..	..	..	*20*	..	..	..	..	..	..	..
From XX and XXI spinal nerves.....	**98**	34	..	..	..	..	7	**7**	14	..	..	..	.	68	8	9	**85**	61.1	..	..	..	..	**6**	100
Fibres from XX through prox. com. branch	89	..	..	..	..	..	7	*7*	..	..	..	..	..	68	8	..	*76*	..	..	..	..	..	*6*	..
Separate nerves from XX and XXI.....	9	..	..	..	..	..	.	..	..	..	..	..	..	.	..	9	*9*	..	..	..	..	..	..	..
From XXI spinal nerve....	**92**	32	..	..	..	..	..	..	..	**6**	100	..	..	..	..	..	..	..	78	8	**86**	100	..	..

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TABLE XI.

ORIGIN OF THE ILIAC NERVES IN THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Iliac branches arise	Totals.		Type I.							Type II.		Type III							Type IV.				Type V.	
			A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
	No.	%	No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
From XIX and XX spinal nerves	**6**	2.1	*2*	..	*2*	..	..	**4**	8	..	..	*1*	..	*1*	..	..	**2**	1.5	..	..	..	..	..	..
As lateral branches of main ventral trunk of each	3	..	1	..	..	..	..	*1*	..	..	..	1	..	1	..	..	*2*	..	..	..	..	..	..	..
From XX, prox. com. br. XIX to XX	3	..	1	..	2	..	..	*3*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
From XX spinal nerve	**77**	27.4	*3*	*4*	*13*	*9*	*3*	**32**	67	..	..	*14*	*20*	*2*	*2*	*2*	**40**	29.1	*4*	..	**4**	4.8	**1**	17
As lat. branch of main ventral trunk	*30*	..	1	..	6	7	2	*16*	..	..	..	1	8	2	2	..	*13*	..	1	..	*1*	..	..	..
As branch of hypogastric	*39*	..	2	4	6	2	1	*15*	..	..	..	12	10	..	..	2	*24*	..	..	..	..	..	..	..
Dorsal origin	*8*	..	..	..	1	..	..	*1*	..	..	..	1	2	..	..	..	*3*	..	3	..	*3*	..	*1*	..
From XX and XXI	**106**	37.7	..	..	*6*	..	*4*	**10**	21	..	..	*10*	*4*	*63*	*6*	*5*	**88**	59.8	*1*	*2*	**3**	3.6	**5**	83
As branches of hypog. of XX and of ing. of XXI	*20*	..	..	..	5	..	..	*5*	..	..	..	9	4	..	..	1	*14*	..	1	..	*1*	..	..	..
As lat. branch of XX and of hypog. of XXI	*10*	..	..	..	..	..	..	..	..	..	..	..	..	3	..	4	*7*	..	..	2	*2*	..	*1*	..
As branch of hypog., (prox. branch XX to XXI)	*69*	..	..	..	..	..	4	*4*	..	..	..	1	..	55	6	..	*62*	..	..	..	..	..	*3*	..
As branch of ing. (prox. branch XX to XXI)	*2*	..	..	..	1	..	..	*1*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	*1*	..
Dorsal origin	*5*	..	..	..	..	..	..	..	..	..	..	..	..	5	..	..	*5*	..	..	..	..	..	..	..
From XXI spinal nerve	**92**	32.7	..	..	..	*2*	..	**2**	4	**6**	..	..	*3*	*2*	..	*2*	**7**	5.1	*71*	*6*	**77**	..	..	..
As branch of hypogastric	77	..	..	..	..	..	..	..	..	*5*	..	..	..	..	..	2	*2*	..	64	6	*70*	..	..	..
As branch of inguinal	1	..	..	..	..	1	..	*1*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
As branches of hypog. and of ing.	6	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	*2*	..	4	..	*4*	..	..	..
Dorsal origin	8	..	..	..	..	1	..	*1*	..	*1*	..	..	3	..	..	..	3	..	3	..	*3*	..	..	..

TABLE XII.

ORIGIN OF THE INGUINAL NERVE IN THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Inguinal nerve arises.	Totals.		Type I.							Type II.		Type III.							Type IV.				Type V.	
	No. of instances.	Per cent.	A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
			No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
From XX spinal nerve	*10*	3.5	4	6	..	..	..	*10*	20	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
As a separate trunk....	6	..	2	4	..	..	..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
In common trunk with hypog. to iliac crest..	2	..	1	1	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
In common trunk with hypog. to external ring	2	..	1	1	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
From XX and XXI spinal nerves.....	*110*	38.3	..	..	21	..	..	*21*	42	..	..	27	..	58	..	..	*85*	61	..	..	..	..	*4*	67
As a separate trunk....	95	..	..	..	21	..	..	21	..	..	..	24	..	46	..	..	70	..	..	..	..	..	4	..
In common trunk with hypog. to iliac crest..	13	..	..	..	..	..	..	..	..	..	..	2	..	11	..	..	13	..	..	..	..	..	..	..
In common trunk with hypog. to external ring	2	..	..	..	..	..	..	..	..	..	..	1	..	1	..	..	2	..	..	..	..	..	..	..
From XXI spinal nerve ...	*148*	51.5	..	..	..	9	7	*1.6*	32	*5*	83	..	27	..	8	8	*43*	31	74	8	*82*	95	*2*	33
As a separate trunk....	123	..	..	..	..	8	7	15	..	3	..	..	27	..	8	7	42	..	55	6	61	..	2	..
In common trunk with hypog. to iliac crest..	21	..	..	..	..	1	..	1	..	1	..	..	..	..	..	1	1	..	17	1	18	..	..	..
In common trunk with hypog. to external ring	4	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	2	1	3	..	..	..
Place taken by genital branch of genito-crural.....	*19*	6.6	1	..	..	2	..	*3*	6	*1*	17	..	..	10	..	1	*11*	8	4	..	*4*	5	..	..

TABLE XIII.

TYPES OF ORIGIN AND DISTRIBUTION OF GENITO-CRURAL NERVES IN VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Type of Distribution.	Totals.		Type I.							Type II.		Type III.							Type IV.				Type V.	
			A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
	No.	%	No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
GENITO-CRURAL NERVES IN COMMON TRUNK UNTIL NEAR POUPART'S LIGAMENT	**154**	61.6	3	4	13	4	2	**26**	59	**5**	100	16	8	33	4	7	**68**	59	49	4	**53**	69	**2**	25
Crural branch emerges in lateral region	12	..	1	1	..	1	..	*3*	..	..	..	2	..	3	..	..	*5*	..	4	..	*4*	..	..	..
" " " in middle "	35	..	1	2	..	..	1	*4*	..	*2*	..	3	2	7	1	3	*16*	..	12	1	*13*	..	..	..
" " " in median "	10	..	..	..	..	..	1	*1*	..	*1*	..	1	..	5	..	2	*8*	..	..	..	..	..	..	..
Genital branch emerges in lateral "	12	..	1	1	..	1	..	*3*	..	..	..	2	..	4	..	..	*6*	..	3	..	*3*	..	..	..
" " " in middle "	33	..	..	2	..	..	1	*3*	..	*2*	..	3	2	7	..	4	*16*	..	11	1	*12*	..	..	..
" " " in median "	12	..	1	..	..	..	1	*2*	..	*1*	..	1	..	4	..	1	*6*	..	3	..	*3*	..	..	..
TWO OR MORE TRUNKS.																								
Separate genital and crural branches	**36**	14.4	..	..	3	2	1		14	..	..	5	6	7	..	..	**18**	15	9	1	**10**	13	**2**	25
Genital emerges, lateral region	15	..	..	..	2	2	1	*5*	..	..	..	..	3	3	..	..	*6*	..	2	1	*3*	..	*1*	..
" " middle "	3	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	*2*	..	1	..	*1*	..	..	..
" " median "	2	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	*2*	..	..	..	..	..	..	..
Crural emerges, lateral region	4	..	..	..	1	..	1	*2*	..	..	..	..	..	2	..	..	*2*	..	..	..	..	..	..	..
" " middle "	11	..	..	..	1	2	..	*3*	..	..	..	..	3	1	..	..	*4*	..	2	1	*3*	..	*1*	..
" " median "	5	..	..	..	..	..	..	..	..	..	..	..	2	2	..	..	4	..	1	..	1	..	..	..
Common genito-crural trunk and extra genital	**16**	6.4	..	1	1	..	..	**2**	4.5	..	..	2	2	9	..	..	**13**	11	..	..	..	..	**1**	12.5
Branches from common trunk emerge, lateral region	2	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	*1*	..	1	..	*1*	..	..	..
" " " " " middle "	11	..	..	..	1	1	..	*2*	..	..	..	..	1	6	..	..	*7*	..	2	..	*2*	..	..	..
" " " " " median "	2	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	*2*	..	..	..	..	..	..	..
Genital branch emerges, lateral region	12	..	..	..	1	..	..	*1*	..	..	..	..	1	7	..	..	*8*	..	3	..	*3*	..	..	..
" " " middle "	2	..	..	..	..	1	..	*1*	..	..	..	..	..	1	..	..	*1*	..	..	..	..	..	..	..
" " " median "	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	*1*	..	..	..	..	..	..	..
Common genito-crural trunk and extra crural	**25**	10.0	2	..	2	..	..	**4**	9	..	..	3	..	4	3	..	**10**	9	9	..	**9**	12	**2**	25
Branches from trunk emerge, lateral region	4	..	2	..	..	..	..	*2*	..	..	..	..	..	..	..	..	..	..	*1*	..	1	..	*1*	..
" " " middle "	5	..	..	..	..	..	..	..	..	..	..	1	..	3	..	..	*4*	..	1	..	*1*	..	..	..
" " " median "	2	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	*1*	..	1	..	*1*	..	..	..
Crural trunk emerges, lateral region	3	..	1	..	..	..	..	*1*	..	..	..	..	..	1	..	..	*1*	..	..	..	..	..	*1*	..
" " " middle "	5	..	1	..	..	..	..	*1*	..	..	..	..	..	1	..	..	*1*	..	3	..	*3*	..	..	..
" " " median "	6	..	..	..	..	..	..	..	..	..	..	2	..	1	..	..	*3*	..	3	..	*3*	..	..	..
Merely a genital nerve, emerges, lateral region	**2**																							
" " " " middle "	**1**	1.2	1	1	..	..	..	**2**	4.5	..	..	..	..	..	..	..	..	..	1	..	**1**	1	..	..
" " " " median "	—																							
Merely a crural nerve, " lateral "	**2**				1	1		**2**	4.5															
" " " " middle "	**14**	6.8	..	..	1	1	..	**2**	4.5	..	..	1	1	2	1	2	**7**	6	4	..	**4**	5	**1**	12.5
" " " " median "	**1**																							
Total	250	..	..	..	..	..	..	44	..	5	..	..	..	..	..	..	116	..	..	..	77	..	8	..

TABLE XIV.

ORIGIN OF THE LATERAL CUTANEOUS NERVE OF THE THIGH IN THE VARIOUS TYPES OF DISTRIBUTION OF THE BORDER-NERVES.

Lateral cutaneous nerve arises	Totals.		Type I.							Type II.		Type III.							Type IV.				Type V.	
	No. of instances.	Per cent.	A	B	C	D	E	Total No.	Per cent.	Total No.	Per cent.	A	B	C	D	E	Total No.	Per cent.	A	B	Total No.	Per cent.	Total No.	Per cent.
			No.	No.	No.	No.	No.					No.	No.	No.	No.	No.			No.	No.				
From XX, XXI, and XXII spinal nerves	*113*	39	4	5	11	9	4	*33*	66	*4*	67	17	11	19	3	3	*53*	38	20	2	*22*	25	*1*	17
From XXI, XXII, and XXIII spinal nerve	*122*	43	1	1	7	1	3	*13*	26	..	..	5	8	36	3	6	*58*	42	44	4	*48*	56	*3*	50
From main trunk of anterior crural	*52*	18	..	..	3	1	..	*4*	8	*2*	33	5	8	13	2	..	*28*	20	14	2	*16*	19	*2*	33
Totals	*287*		5	6	21	11	7	*50*		*6*		27	27	68	8	9	*139*		78	8	*86*		*6*	

THE DEVELOPMENT OF THE VENA CAVA INFERIOR.

BY

FREDERIC T. LEWIS, A. M., M. D.

From the Embryological Laboratory, Harvard Medical School.

WITH 11 TEXT FIGURES AND TWO DOUBLE COLORED PLATES.

In a course of lectures on the problems of embryology, Prof. C. S. Minot demonstrated that the current descriptions of the embryonic vena cava inferior are inadequate, if not actually erroneous. Under his direction, the rabbit embryos of the Harvard Embryological Collection have been examined in order to revise Hochstetter's work, already once repeated by Zumstein. The results of this third investigation of the rabbit's cava inferior justify a new description of the vein, illustrated by lithographs, a gift from the Elizabeth Thompson Science Fund.

In rabbit embryos of about ten days, the abdominal veins are absolutely symmetrical. On either side of the intestinal canal runs an omphalo-mesenteric vein, which unites with the umbilical vein from the somatopleure of the corresponding side just before joining the duct of Cuvier. (See Hochstetter, 93, p. 546, Fig. 1.) For reasons which have not been explained this system becomes asymmetrical, and normally is predominant on the right side. On the 12th day the venous orifice of the heart is on the right; the right umbilical and right omphalo-mesenteric veins are larger than the left;[1] and the vessels on the left side are forming a new channel, the ductus venosus Arantii, which conveys their blood directly across the liver to the right auricle.

The liver develops from the ventral wall of the intestine by sending its tubules into, and thus subdividing the omphalo-mesenteric veins. This condition was noted by Hochstetter (93, p. 546) and others, and has since been fully described by Minot (00, pp. 197-202), who named the small venous subdivisions "sinusoids."

As the venous channels become predominant on the right side, the liver consequently develops more rapidly there and becomes a right-

[1] Although the right umbilical vein, in the early part of the 12th day, is larger than the left, and may be described as "colossal," the *left* umbilical vein is the one which persists throughout embryonic life.

sided organ. Veins and liver combine to push the stomach toward the left. Fig. 1, a frontal section of a 5 mm. rabbit, shows clearly the barrier formed by the veins and liver, with the consequent forcing of the stomach to the left. It may confidently be assumed that had the originally symmetrical veins persisted on the left side, stomach, liver, and later the vena cava would shift their position, resulting in situs inversus.

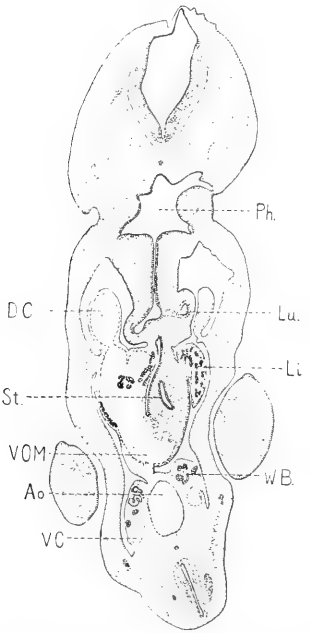


Fig. 1. Rabbit embryo of 5 mm., 12 days. Series 105, section 140. × 25

The position of the vena cava inferior as yet unformed, has been determined by the asymmetrical development of the umbilical and omphalo-mesenteric veins. In the early rabbit stages, there springs from the root of the dorsal mesentery a pair of mesenchymal lobes, one on either side. Traced anteriorly they pass into the mesenchymal anlages of the lungs, the alæ pulmonales of Ravn (**89**, p. 139), which precede the epithelial outpocketings from the œsophagus. These wings are at first quite symmetrical, but with the displacement of the stomach, the fold on the left is obliterated and that on the right enlarges. Fig. 2 is a cross-section of a 5 mm. rabbit. The bulging of the stomach toward the left has caused it to present on that side a smooth rounded surface, but on the right it is irregularly indented and the mesenchymal fold referred to, C. M., becomes accentuated. This fold is destined to contain the inferior cava, and has been called the "mesenteric bridge" by Goette (**75**, p. 818), the "plica venæ cavæ" by Ravn (**89**, p. 140), and the "caval mesentery" by Hochstetter (**93**, p. 564). In later stages the elongated gastric mesentery runs sharply to the left, and from its right side, where it joins the body wall, springs this caval mesentery. As a whole the mesentery is now V-shaped, the left arm or mesogastrium going to the

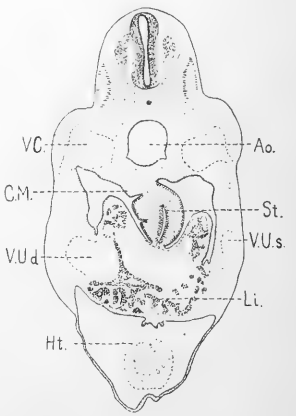
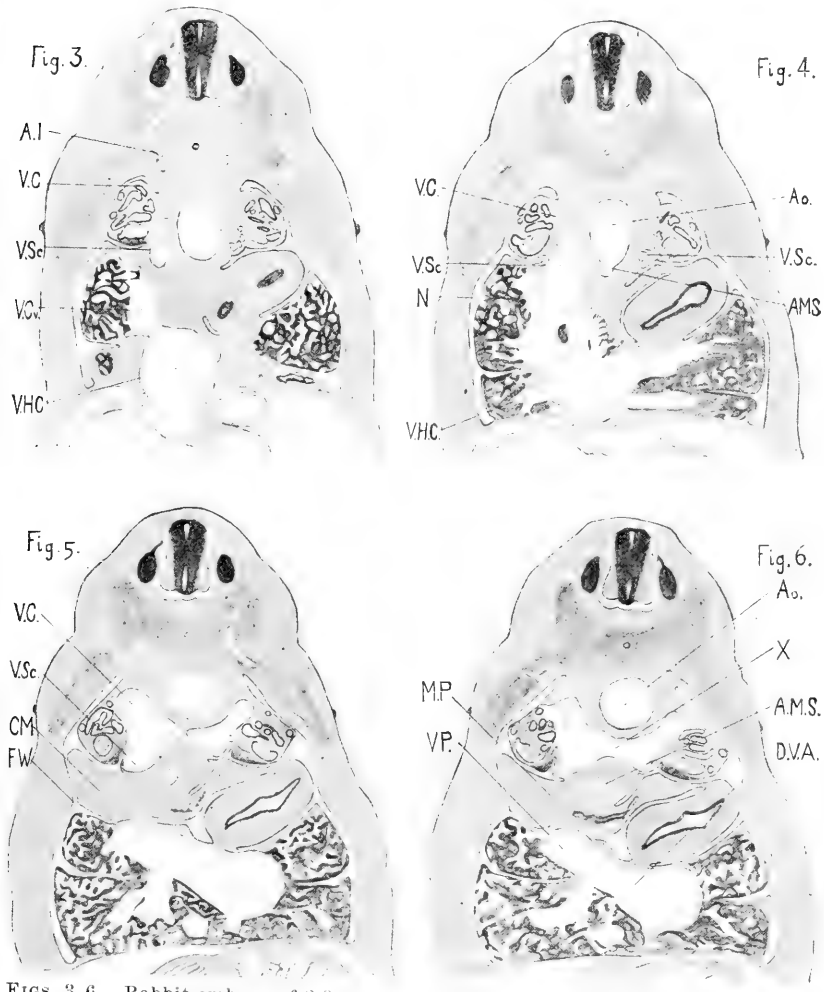


Fig. 2. Rabbit embryo of 5 mm., 12 days. Series 104, section 317. × 25.

stomach, and the right arm or caval mesentery ending freely in the abdominal cavity. The fate of the caval mesentery is briefly this. The part most cephalad is invaded by the right lung, of which it



FIGS. 3–6. Rabbit embryo of 8.8 mm., 13 days. Series 465, sections 256, 246, 217, 215, respectively. × 20.

forms the lobus inferior medialis of Krause (Ravn, **89**, p. 144). Below the diaphragm it meets and unites with the liver. Hepatic tubules grow into it and it becomes a part of the liver. Thus it at once connects the liver with the right dorsal wall and causes the hepatic sinu-

soids to come quite close to the posterior cardinal vein. Still further caudad there is a place where the caval mesentery has not united with the liver, but is free. Between liver and mesentery there is left a long slender passage, the foramen of Winslow.

These relations are illustrated by Figs. 3-6, transverse sections of an 8.8 mm. rabbit. In Fig. 5 the foramen of Winslow, F. W., bounded by caval mesentery and by liver, is seen leading to the lesser omental cavity. Fig. 4 shows the caval mesentery uniting with the liver above the foramen of Winslow; a notch, N, marks the limit of the original hepatic lobe, but the tubules now fill the caval mesentery. At a higher level, as in Fig. 3, the notch becomes obliterated. Below the foramen of Winslow the caval mesentery again unites with the liver, as shown in Fig. 6, but is here rather a "portal mesentery," for it contains the portal vein. Since the relations of this portal mesentery and its connection with the ventral border of the stomach have nothing to do with the cava inferior, its further consideration is reserved for a subsequent paper. The foregoing description has shown how the path for the vena cava is laid out, and why the vein is to be unilateral. We may now consider the development of the vessel itself.

In the embryos of 5 mm. the aorta passes toward the tail as a median unpaired vessel lying dorsal to the root of the mesentery and ventral to the spinal cord. On either side of it runs a posterior cardinal vein, as shown in Fig. 2. The Wolffian bodies are found in the caudad part of the abdomen, ventral to the cardinal veins. The tributaries of the posterior cardinals are the intersegmental veins arising regularly between the dorsal ganglia, and a number of irregularly placed small vessels which come from the mesenchyma in front of the aorta, and from that around the Wolffian tubules. These branches are obscure in the early stages but in an embryo of 6.6 mm. they are plainly seen. Fig. 7 illustrates their arrangement. A vessel is shown passing from the mesentery into the left posterior cardinal vein. The Wolffian body is represented by a knot of coiled tubules. The tributary of the cardinal passes along its median surface, after receiving a branch from its ventral border. Other cardinal branches curve over its dorsal side so that the Wolffian body becomes nearly surrounded by veins. The later intercrescence of Wolffian tubules and cardinal veins has been carefully described by Minot in his paper on sinusoidal circulation (**oo**, pp. 193-197). It is important that the cardinal tributaries may anastomose with one another, and, in front of the aorta, with those of the opposite side. These anastomoses are found in embryos of 7.5 mm.

Beginning with an embryo of this length four stages in the develop-

ment of the veins have been illustrated by reconstruction after the method of His. The drawings are similarly enlarged and arranged in Plates I and II. Each pair of figures represents a single embryo, split in the median plane and laid open, the left half of the embryo lying on the right-hand page, and vice versa. All the blood-vessels involved have been drawn except the median aorta and its median (mesenteric and gastric) branches. Every drawing shows two sets of arteries: 1st, a regularly arranged series of intersegmental arteries, A. I., and 2nd, the irregularly disposed arteries running laterally from aorta to the

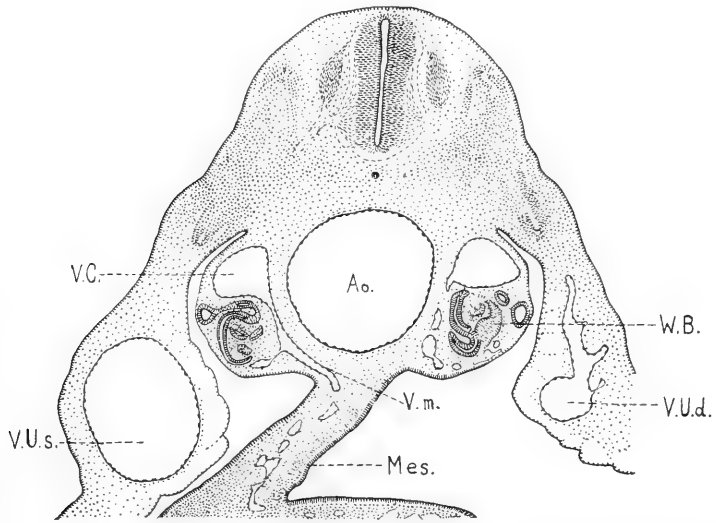


FIG. 7. Rabbit embryo of 6.6 mm., 12 days, 12 hours. Series 460, section 117. × 45. This section should have been reversed to be in the conventional position. The right side is here drawn at the right of the observer.

glomeruli of the Wolffian body. These may be named the mesonephric arteries, A. M.: their position in relation to the veins makes them a most important landmark. The umbilical arteries, A. U., are also indicated. All the arteries are shown as cut across at the position where they leave the aorta.

Figs. 1 and 2, Plate I, picture the condition already described. In this 7.5 mm. embryo the posterior cardinal veins, V. C., pass cephalad to join the anterior cardinals, and then to turn back at a sharp angle and enter the heart. Ventral to the posterior cardinal vein is seen the line of mesonephric arteries, and ventral to these is an anastomosis of cardinal tributaries. This anastomosis forms a new vessel coming from

the cardinal in the caudad region, emptying again into the cardinal anteriorly, and connected with the cardinal all along the line by cross branches running between the mesonephric arteries. This new vessel I would designate as the *subcardinal vein*, Vena subcardinalis. The bilateral symmetry of the veins at this stage is complete. The small vessels from the mesentery, and mesenchyma ventral to the aorta are represented in the drawings, one of them being labelled V. m., and two points of anastomosis with the veins of the opposite side are designated by the letters V. m. x. Thus the subcardinal veins are connected with one another by vessels of small calibre.

In Fig. 1, Plate I, a portion of the liver has been outlined. The portal vein, V. O. M., is shown cut across in the median plane. It passes behind the intestine toward the right and forward through the liver connecting with the ductus venosus, D. V. A., and ending in a vessel known as the vena hepatica communis. This comprehensive name was applied by Hochstetter (93, p. 552) to that trunk passing from the liver to the heart, and formed by the union of hepatic, umbilical, and omphalo-mesenteric veins, to which the inferior vena cava is later added. In the figure a black, partly dotted line marks the limit of the dorsal hepatic lobe, which is filled with sinusoids. Some of these channels, V. c. m., have extended over into the caval mesentery, into which the hepatic cylinders are to follow them.

A large stream of blood traverses the liver through the broad venous spaces unimpeded, whereas the current through the cardinal veins is clogged by the Wolffian tubules. The development of the posterior limbs demands a freer passage, and the formation of the subcardinals may be regarded as the attempt of the cardinal veins to become disentangled from the Wolffian body. Probably the recurrent bend of the duct of the Cuvier is another obstruction to the posterior cardinal system. At all events the right subcardinal and the hepatic sinusoids approach one another and unite, thus forming a new access to the heart. All the component parts of the adult vena cava inferior have now become connected. The new passage is so favorable that it enlarges rapidly. On the left side, the subcardinal can make no connection with the liver, since the stomach has cut off any approach to that organ. There has been no reversal of blood currents on either side, but blood from the lower left area crosses to the right through the anastomoses between the subcardinals. The cardinal system has been tapped by the hepatic.

Figs. 3 and 4, Pl. I, represent a rabbit of 8.8 mm. The subcardinal veins, V. Sc., are now large, and that on the right has connected with the vena hepatica communis. See also the cross section, Fig. 4, p. 231.

It extends for a short distance beyond this connection, as shown clearly in the cross section, Fig. 3. Below the superior mesenteric artery five anastomoses between the subcardinals could be followed, the first of which is lettered X. Fig. 6 of the cross sections passes through the anastomosis X. Just below the superior mesenteric artery a cross connection between subcardinal and cardinal becomes very large and marks an important subdivision of both vessels into superior and inferior parts. The superior portion of the subcardinal enlarges, its inferior division diminishes, and correspondingly the inferior section of the cardinal enlarges, its superior part becomes small. Thus the inferior

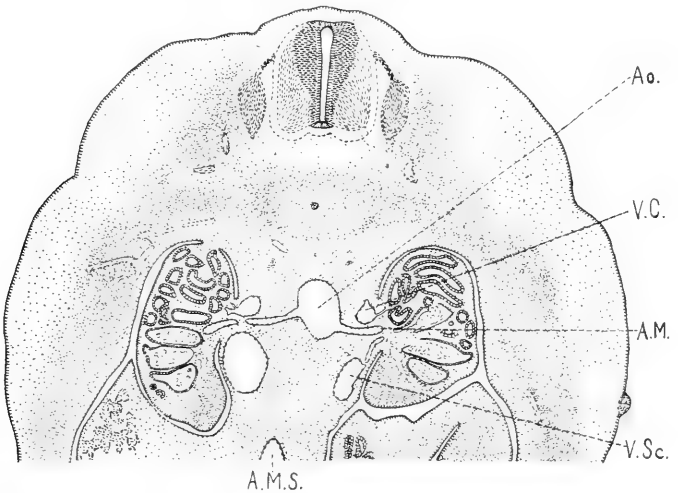


Fig. 8. Rabbit embryo of 11 mm., 14 days. × 28. This series is not one of the Harvard Collection.

part of the cardinal and superior part of the subcardinal are both large, and, by the obliteration of the kink made by the anastomosis between them, become a single straight channel. On the right side they persist as a part of the adult vena cava inferior.

As the superior part of the cardinal vein shrinks in calibre, it loses its continuity as a venous trunk, although its disjoined sections are still connected by the sinusoids of the Wolffian body. Fig. 3, Pl. I, shows the anterior part of the cardinal, V. C. a., separated from the posterior division, V. C. p. On the left side, however, the vessel is still continuous from the pelvis to the duct of Cuvier.

The third reconstruction is from an 11 mm. rabbit, Figs. 5 and 6, Pl. II. The cardinal veins are now both subdivided as just described.

A notable change is in the decreasing importance of the inferior part of the subcardinal veins. Only two of its cross connections remain, of which one, X, has become very large. A cross section, Fig. 8, of this embryo, taken a short distance above this region shows the symmetrical arrangement of the vessels, and the large size of the subcardinals on both sides. The subcardinals lie at the ventral corner of the hilus of the Wolffian body from which they receive tributaries, just as do the cardinals at the dorsal corner. The veins are separated from one another by the mesonephric arteries, a pair of which is seen in the section. At the upper end of the veins, on either side, cardinal and subcardinal anastomose in condensed mesenchyma probably connected with the suprarenal anlage.

In the rabbit of 8.8 mm., the kidney on either side was situated in front of the iliac artery, as described by Hochstetter, and beautifully drawn in his Fig. 18 of Pl. XXII (93). As it develops, it drops back over the artery and falls between the cardinal vein and the aorta, or even directly upon the cardinal. It may split the cardinal vein so as to form a loop, as figured by Hochstetter, but I have not seen any complete loop. In Fig. 6, Pl. II, the position of the kidney is indicated by R. A portion of the cardinal vein receiving two intersegmental veins has been separated from the main trunk and pushed dorsad. On the right side of the same embryo, Fig. 5, Pl. II, the vein was not divided, but the kidney had distorted the course of two intersegmental veins. The main cardinal stem bends rather sharply outward around the obstructing kidney and so comes to lie on the outer side of the lower end of the Wolffian body.[1] The ureter is now on the median side of this large trunk. From the shattered inner pieces of the cardinal vein, or from new offshoots of the main stem, a venous connection forms on the median side of the ureter. Such a loop is seen in Fig. 5, the letter U marking the passage for the ureter. This new median arm of the loop is in line with the main vessel; it enlarges and becomes a part of the cardinal trunk. The vein has again become straight, but the ureter has been transferred from its inner to its outer side. The outer arm of the loop becomes smaller, and its caudad portion is divided into many sinusoids. It then appears as a large branch of the cardinal vein, entering it from the dorsal border of the Wolffian body. Thus it forms the Urnierenvene of Hochstetter (93, p. 583). The ureter remains in the loop and passes, therefore, ventral to the iliac artery, external to the

[1] In pig embryos of 12.0 mm. the main cardinal vessel passes to the outer side of the caudal end of the Wolffian body uninfluenced by the renal anlage.

cardinal vein, and dorsal to the Urnierenvene. My examination of these renal relations confirms the observations of Hochstetter (88 and 93) in almost every particular.

Figs. 7 and 8, Pl. II, from a rabbit of 14.5 mm., show at Y the new cross connection between the cardinal veins in their pelvic portion. The kidney lies behind the Wolffian body, the hilus of which it compresses, as shown in Fig. 9. The renal vein is a branch of the cardinal at the level of the large anastomosis of that vessel with the subcardinal. In the pelvic part of its course the cardinal receives the vena lumbalis

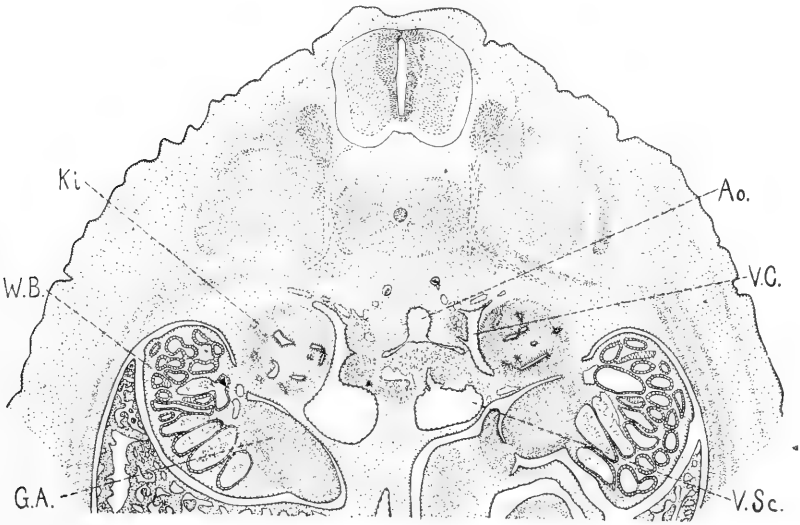


Fig. 9. Rabbit embryo of 14.5 mm., 14 days, 18 hours (?). Series 143, section 827. × 30.

transversa posterior. This is a large irregular vessel in the body wall, connected with one or two intersegmental veins, and suggesting the trunk which was split off by the kidney shown in Fig. 4, Pl. I. The intersegmental veins were easily followed in younger and in older embryos, but at this stage their connections with the cardinal are very obscure. This may be due to distortion caused by the migration of the renal artery. The superior section of the cardinal receives a large transverse lumbar vein, two intersegmental veins, and small vessels from mesenchyma in the suprarenal region. On the right side a considerable area fuses with the subcardinal and is incorporated in the vena cava. The mesonephric arteries become obliterated. In a rabbit

of 21 mm. a single one of them remained on either side above the anastomosis X. The relations of the vessels in this region are shown in Fig. 9, the section on the right side passing through the fused subcardinal and cardinal. The inferior sections of the subcardinal veins no longer receive branches from the Wolffian bodies, but appear as large spaces in the mesentery, conspicuously empty of corpuscles, though the adjoining veins are full of them. The mesenchyma in front of the aorta below the anastomosis X is permeated by these spaces, as shown in Fig. 10. Mere strands of mesenchyma separate them from one another and the adjoining veins. Their fate is unknown, but they recall the description by Sala, oo, of the anlages of lymphatic hearts in the chick. They pass into slender vessels, empty of corpuscles, which extend some distance cephalad from the anastomosis X. They appear in the position previously occupied by the lower part of the subcardinal, and at the time when those vessels disappear. Therefore they may be subcardinal derivatives.

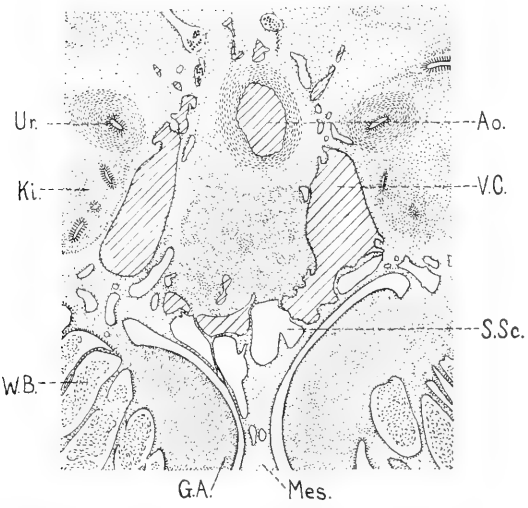


FIG. 10. Rabbit embryo of 14.5 mm., 14 days, 18 hours (?). Series 143, section 857. × 45. The vessels shaded with lines contain corpuscles; the others are empty.

On the left side, the superior part of the subcardinal vein begins as a considerable branch from the Wolffian body, uniting with another from the suprarenal area. It receives vessels from both structures along its course. The size of this part of the left subcardinal in a 21 mm. rabbit is shown in Fig. 11, drawn with the same magnification as Fig. 8, Pl. II. The suprarenal body is found between the cardinal and subcardinal veins which lie very close to it, the cardinal at the dorsal

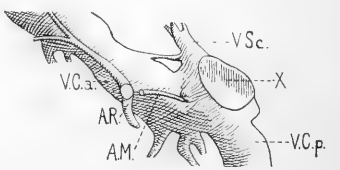


FIG. 11. Reconstruction from rabbit embryo of 21 mm., 17 days. Series 164. × 25.

surface and the subcardinal ventrally. The suprarenal body extends far beyond the traceable branches of the subcardinal vein. In a 29 mm. rabbit the relations were similar. The very small and short subcardinal vein came from the suprarenal body, from which possibly the cardinal also received some small branches. They could not definitely be followed. In Hochstetter's description the left subcardinal is named the left suprarenal vein from the first. It is quite probable that the subcardinal may become the adult suprarenal, though I shall not consider it an established fact until still older embryos have been examined. Kollmann (98, p. 477) writes that it becomes "bis auf unbedeutende Gefässe rudimentär."

The posterior section of the left cardinal vein becomes divided either above the Urnierenvene, or below it, or below the transverse lumbar vein. Hochstetter (93, pp. 585-586), gives figures of the three resulting conditions in the adult. In the 21 mm. rabbit the division had occurred below the transverse lumbar vein, as is usual; the 29 mm. specimen had divided above that vein. The Urnierenvene becomes the spermatic vein of the adult; and the remaining part of the posterior section of the left cardinal, into which it flows, is called the ascending lumbar vein. This lumbar vein terminates in the renal vein, the old anastomosis extending from the cardinal through the subcardinal to the opposite side. No later changes of importance occur in these veins.

The present inaccurate text-book accounts of the development of the vena cava inferior have justified the reiteration of many well established observations. Schäfer, in Quain's Anatomy, illustrates a very brief description by diagrams from Kölliker. These figures, published in Kölliker's Grundriss in 1884 (p. 404, Fig. 276) among other errors represent the cava as a vessel separate from the cardinals to the common iliacs. Schultze in 1897 (p. 406, Fig. 357) replaced these diagrams by a modification of those of Hertwig, whose faulty figures have enjoyed great popularity. (See Hertwig, 00, p. 350, Fig. 315.) Kollmann (98, p. 470, Fig. 292 A) gives the only accurate diagram of the early vena cava inferior which I have seen. His figure agrees with the description by Hochstetter of the vein on the left side, symmetrical with the vena cava on the right, but Kollmann is misled in stating that the vena cava "*setzt sich* in Verbindung" with the cardinal veins. In this he follows Hochstetter's earlier description (93, p. 569).

"The posterior vena cava passes over from the liver into the caudal continuation of the caval mesentery (into which small branches from the hepatic venous network also enter), and thence may be followed, in the youngest stage in which I saw the cava, a short distance further

toward the median side of the right Wolffian body. In the next older stage the posterior vena cava passes through the caval mesentery and then puts itself on the median side of the Wolffian body, along which it may be followed for a considerable distance beyond the place of origin of the superior mesenteric artery. Moreover, there is a vein on the left side quite similar in position to the caval trunk on the right, which extends as far caudad as the latter, and begins at the level at which the vena cava meets the right Wolffian body. It is joined to the vessel on the right by two or three weak cross connections. These roots of the posterior cava are united with the cardinal vein only by very weak vessels which should be reckoned as capillaries."

This description has been taken to mean that the vena cava inferior developed downward from the liver, that the symmetrical vein on the left was a branch of it, and that this system acquired its connection with the cardinals. Although his language is somewhat vague, this, I believe, is what Hochstetter meant. On p. 602 he describes a human embryo in which the symmetrical veins (subcardinals) were apparently separate from one another. In a significant foot note Hochstetter says that cross connections may have been present but invisible because empty, "otherwise the left vessel must be regarded as an independent anlage." Grosser (01, p. 362) describes a similar condition in bats. "The right cardinal vein stands already in broad connection with the posterior vena cava which continues beyond this anastomosis along the median side of the Wolffian body. A left *Hohlvenenanlage* symmetrical with the right is present, and joined to the right by one (or two?) almost capillary channels. Moreover, this vessel is united with the left posterior cardinal by slender vessels, but this union plays no great rôle." Nevertheless, I consider these connections to be important and primary, the longitudinal anastomosis forming next, and, finally, on the right side, the union with the liver which has invaded the caval mesentery. Zumstein (98, pp. 311-312) gives a more accurate description than Hochstetter. In the mole of 3 mm. he found "on both sides, median and ventral to the cardinals, small venous passages which united with the cardinal veins. Those on the right could be followed to the hepatic vessels. This condition differs from that of younger stages in possessing a clear connection between the right cardinal vein and the hepatic vessels. In the liver itself there is no clear passage which can be designated as the vena cava inferior." Zumstein did not appreciate the importance of these observations, which are illustrated by crude figures. He concluded his paper by disputing with Hochstetter regarding the spermatic veins and was told in reply (Hochstetter, 98, p. 517) that he had "brought to light no new fact regarding the development of the rabbit's inferior cava."

That the plan of development described for the rabbit is of wide application is probable. Grosser has found it in bats, Zumstein in the mole, Hochstetter and Kollmann have indicated it in human embryos. I have found a similar arrangement in pigs, and note that the reconstruction of the pig's veins by Minot (98, Pl. I, Fig. 5) is incomplete. Dr. Minot has pointed out to me in the original reconstruction several sectioned areas in the veins, omitted in the figure. A more complete drawing of these vessels is to appear in his "Text-book of Embryology."

Finally a paragraph concerning nomenclature! The vena cava inferior is a compound vessel belonging to the adult rather than to the embryo. It consists of a part of the heart, then in turn, parts of the vena hepatica communis, dilated sinusoids of the liver, part of the right subcardinal vein, and a section of the right posterior cardinal vein. It has been the custom of certain embryologists to give the name of the whole to one of its parts, namely to the subcardinal portion, and even to charge with ignorance those who called other sections of the adult vessel the vena cava. I agree with Dwight (01, p. 29) that it is quite accurate to speak of the "cava below the diaphragm" or above the diaphragm.

Summary.

The persistence of the right umbilical and right omphalo-mesenteric veins causes the stomach to be pushed to the left side and the liver to become predominant on the right.

This displacement of the stomach causes the left mesenteric fold, continuous with the ala pulmonalis, to disappear; but the fold on the right, the caval mesentery, enlarges. It fuses with the liver, becomes invaded by hepatic tubules and made a part of the right dorsal hepatic lobe. Thus it causes the hepatic vessels to lie near the posterior cardinal vein.

Small vessels from the mesentery pass into the cardinals. They anastomose in front of the aorta with the vessels of the other side. They form a longitudinal anastomosis parallel with the cardinal vein, with which it is connected by numerous short veins, and from which it is separated by a line of mesonephric arteries. This longitudinal vessel connected with the cardinal vein at both ends, and bilaterally symmetrical in its early stages is the *subcardinal vein*.

The cross connections between the subcardinal veins give place to a single large cross anastomosis caudad to the origin of the superior mesenteric artery. Above this anastomosis the right subcardinal connects with the liver and rapidly enlarges; the left subcardinal becomes very

small—Hochstetter says that it forms the left suprarenal of the adult. Below the anastomosis the subcardinals cease to exist as veins; they may persist as lymphatic spaces.

The vena cava inferior is a compound vessel composed of parts of the heart, the vena hepatica communis, the hepatic sinusoids, the upper part of the right subcardinal, and the lower part of the right cardinal vein.

LITERATURE CITED.

Dwight, Thomas, 01.—What constitutes the inferior vena cava? Anat. Anz., Vol. XIX, pp. 29-30.

Goette, Alexander, 75.—Die Entwickelungsgeschichte der Unke. Leipzig. pp. 1-196.

Grosser, Otto, 01.—Zur Anat. und Entw. des Gefässsystemes der Chiropteren. Anat. Hefte, Abt. 1, Vol. XVII, pp. 203-424.

Hertwig, Oscar, 00.—Die Elemente der Entwicklungslehre des Menschen. Jena. pp. 1-406.

Hochstetter, Ferdinand, 88.—Ueber den Einfluss der Entwickelung der bleibenden Nieren auf die Lage des Urnierenabschnittes der hinteren Cardinalvenen. Anat. Anz., Vol. III, pp. 938-940.

Hochstetter, Ferdinand, 93.—Beiträge zur Entwicklungsgeschicte des Venensystems der Amnioten. III Säuger. Morph. Jahrb., Vol. XX, pp. 543-648.

Hochstetter, Ferdinand, 98.—Bemerkungen zu Zumsteins Arbeit. Anat. Hefte, Abt. 1, Vol. X, pp. 511-517.

Kölliker, Albert, 84.—Grundriss der Entwickelungsgeschichte des Menschen. Leipzig. 2nd ed., pp. 1-454.

Kollmann, J.—98.—Lehrbuch der Entwickelungsgeschichte des Menschen. Jena. pp. 1-658.

Minot, Charles S., 98.—On the veins of the Wolffian body in the pig. Proc. Bost. Soc. of Nat. Hist., Vol. XXVIII, pp. 265-274.

Minot, Charles S., 00.—On a hitherto unrecognized form of blood circulation without capillaries in the organs of Vertebrata. Proc. Bost. Soc. of. Nat. Hist., vol. XXIX, pp. 185-215.

Ravn, Edvard, 89.—Ueber die Bildung der Scheidewand zwischen Brust- und Bauchhöhle in Säugethierembryonen. Arch. f. Anat. u. Entw. 1889, pp. 123-154.

Sala, Luigi, 00.—Sullo sviluppo dei cuori linfatici e dei dotti toracici nell' embrione di pollo. Ric. fatte nel Lab. di Anat. norm. d. R. Univ. di Roma, Vol. VII, pp. 263-296.

Schäfer, Edward A., 90.—Quain's Anatomy, 10th ed., Vol. I, part 1, pp. 1-169.

Schultze, Oscar, 97.—Grundriss der Entwicklungsgeschichte des Menschen. Leipzig. pp. 1-468.

Zumstein, J., 98.—Uber die Entwickelung der Vena cava inferior bei dem Maulwurfe und bei dem Kaninchen. Anat. Hefte, Abt. 1, Vol. X, pp. 309-342.

EXPLANATION OF PLATES.

PLATE I.

Figs. 1 and 2. Reconstruction from a rabbit embryo of 7.5 mm., 12 days, 12 hours. Series 454. × 25.

FIGS. 3 and 4. Reconstruction from a rabbit embryo of 8.8 mm., 13 days. Series 465. × 25.

PLATE II.

FIGS. 5 and 6. Reconstruction from a rabbit embryo of 11.0 mm., 14 days. This series is not one of the Harvard collection. × 25.

FIGS. 7 and 8. Reconstruction from a rabbit embryo of 14.5 mm., 14 days, 18 hours (?). Series 143. × 25.

LETTERING OF PLATES AND FIGURES.

The first and last of the spinal ganglia represented in the plates are numbered, the first cervical ganglion being counted 1.

A. I., intersegmental artery. In each plate only the first and last have been lettered.

A. M., mesonephric artery. In each plate only one of several has been lettered.

A. M. S., superior mesenteric artery.

Ao., aorta

A. R., renal artery.

A. U., umbilical artery.

C. M., caval mesentery.

D. C., duct of Cuvier.

D. V. A., ductus venosus Arantii.

F. W., foramen of Winslow.

G. A., genital anlage.

Ht., heart.

Ki., kidney.

Li., liver. (In Plate I the outline of its right dorsal lobe.)

Lu., anlage of lung.

Mes., mesentery.

M. P., portal mesentery, that part of C. M. caudad to the F. W.

N., notch between right dorsal hepatic lobe and C. M.

Ph., pharynx.

R., position of renal anlage.

Sp. C., spinal cord.

S. Sc., subcardinal spaces.

St., stomach.

U., venous loop through which the ureter passes.

U. V., Urnierenvene (Hochstetter).

Ur., ureter.

V. C., posterior cardinal vein.

V. C. a., posterior cardinal vein, anterior division.

V. C. p., posterior cardinal vein, posterior division.

V. c. m., vein extending into C. M.

V. Cv., vena cava, hepatic portion.

V. H. C., vena hepatica communis.

V. L. T. P., posterior transverse lumbar vein.

V. m., small vein running into mesentery. In the plate similar ones, caudad, are not lettered.

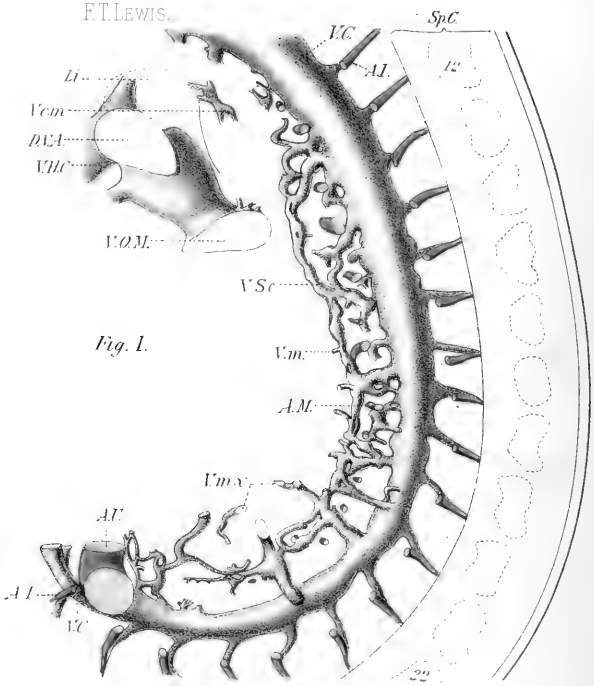
V. m. x., vein running into mesentery and anastomosing with those of the opposite side.

V. O. M., omphalo-mesenteric vein. (Portal vein.)

V. P., portal vein, entering the liver from the M. P.

V. R., renal vein.

V. Sp., subcardinal vein.
V. Sc. i., subcardinal vein, inferior division.
V. Sc. w., subcardinal vein, branch from Wolffian body.
V. U. d., right umbilical vein.
V. U. s., left umbilical vein.
W. B., Wolffian body.
X., cross connection between subcardinals.
Y., cross connection between posterior cardinals.


SpC
VC
AI
12
Li
Vcm
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V.Sc
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A.M.
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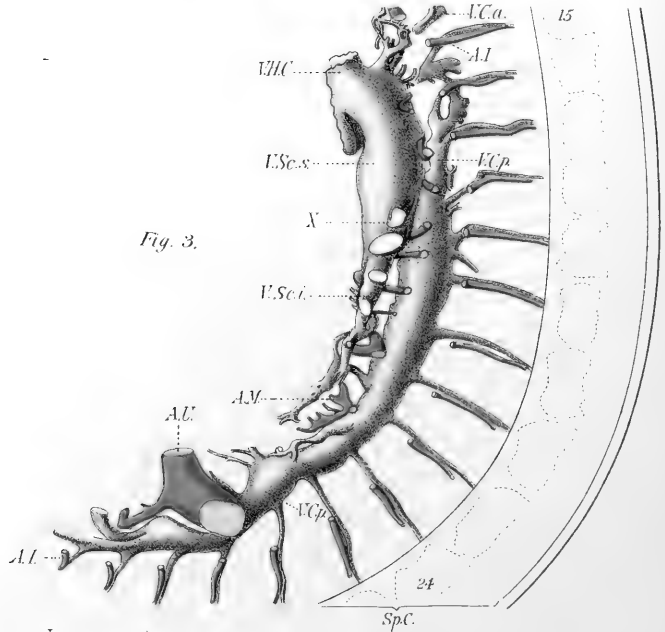
A.U
A.I
VC
22

Fig. 1.


V.Ca.
15
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VHC
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SpC.

Fig. 3.

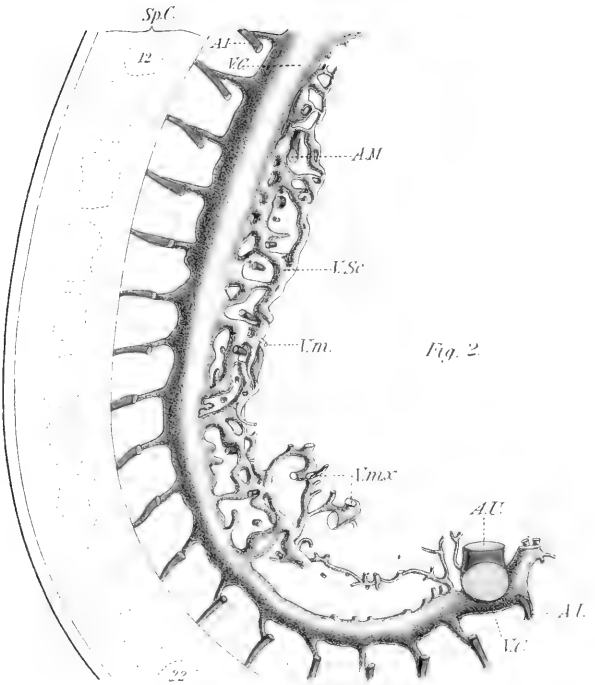


Fig. 2.

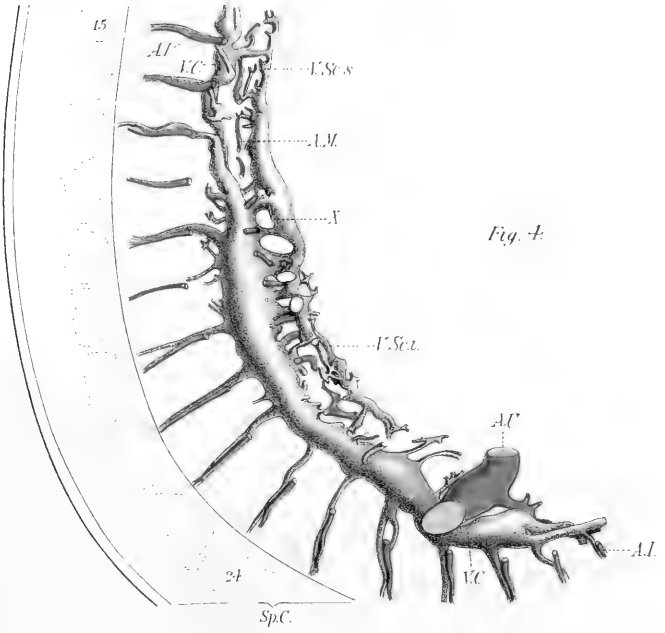
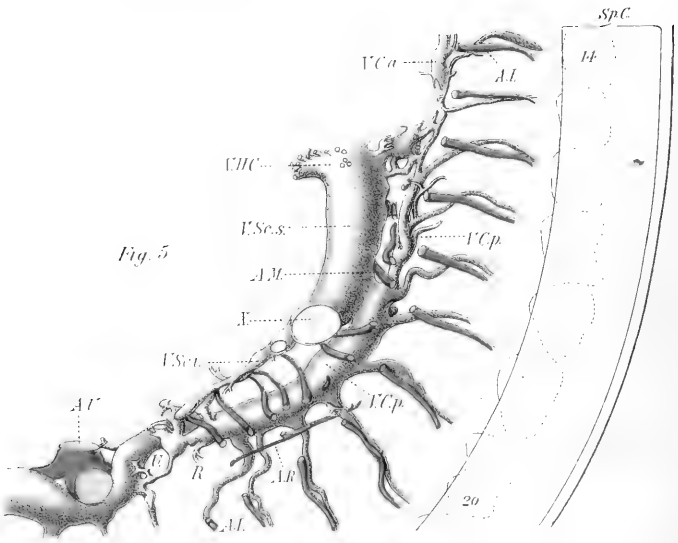


Fig. 4.

S. Metcalf, lith. Boston.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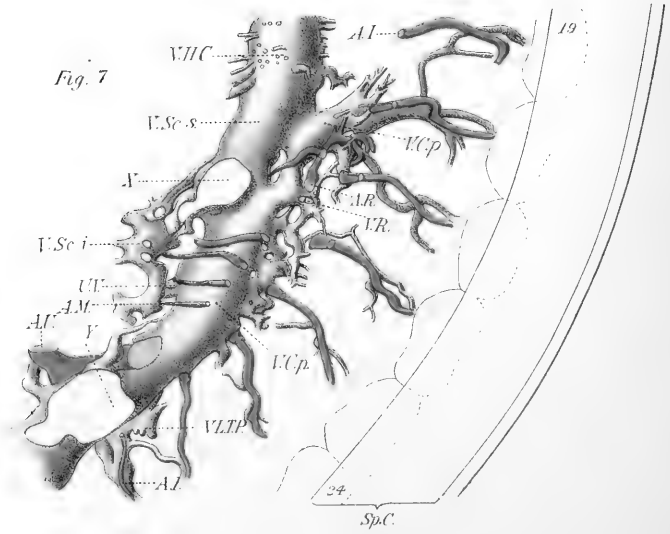
20

Fig. 5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Sp.C.

Fig. 7

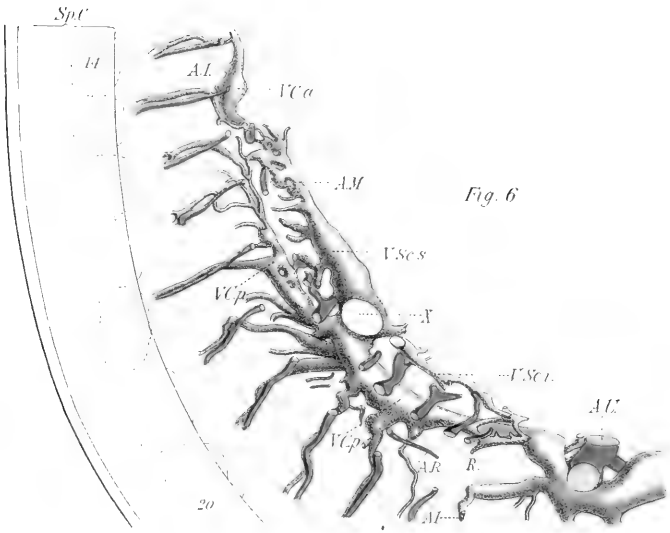

Fig. 6

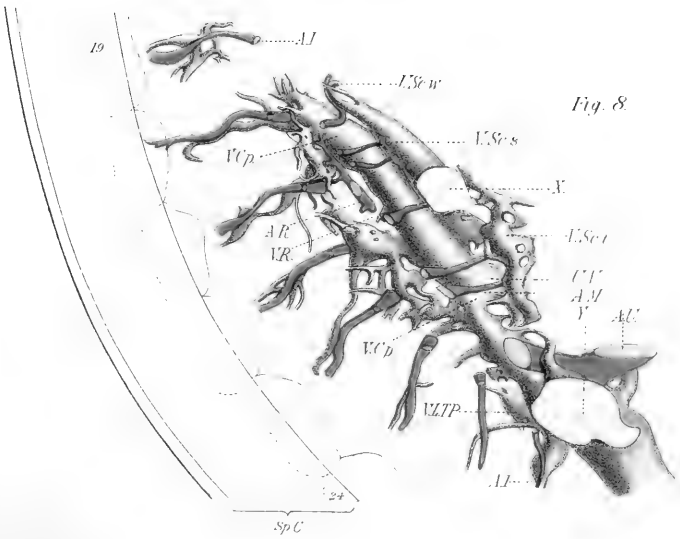

Fig. 8

B. Meisel, lith. Boston.

NOTES ON THE WOLFFIAN BODY OF HIGHER MAMMALS.

BY

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From the Anatomical Laboratory, Johns Hopkins University.

WITH 17 TEXT FIGURES.

In studying the Wolffian body of human and pigs' embryos, certain facts were arrived at which will be set down in the following order:

1. Methods of study and material.
2. Tubular system of Wolffian body in human embryos.
3. Tubular system of Wolffian body in pigs' embryos with a description of a wax reconstruction showing the course of the tubules.
4. Blood-vessels of Wolffian body of pig's embryo.
5. Relation of the tubular systems of the testis and the Wolffian body.

1. METHODS OF STUDY AND MATERIAL.

A large number of pigs' embryos were studied, varying in length from 8 mm. to 200 mm. Since a fresh supply of these could be obtained at any time, it was not difficult to make a considerable number of injections and preparations with various methods. The human embryos were available through the kindness of Professor Mall, and free use was made of the large collection which he possesses.

A very instructive method in the study of the tubular system consisted in the injection of the organ through the allantois with a colored solution. Various injection masses were made use of, but the most satisfactory proved to be the saturated aqueous solution of Berlin blue, and the ordinary carmine gelatin mass. Gelatin in which cinnabar or lamp-black granules were suspended had the disadvantage of being less transparent. Double injections were also made with carmine gelatin for the tubules and Berlin blue for the blood-vessels. In the Wolffian body there is no difficulty in distinguishing veins from arteries with a general vascular injection. Double injections of the vessels, however, were also made to supplement the general ones.

In forcing fluid into the tubules of the Wolffian body it is necessary to inject either through the allantois or the cloaca. In small embryos it is easier to tie off the cloaca below the entrance of the Wolffian duct, and fill the allantois with the injection mass. Then by gently squeezing the allantois between the fingers the fluid can be forced slowly into the tubules of the Wolffian body. In older embryos the cannula can be placed in the cloaca, and the allantois tied off. Where possible this is the best method, because the soft gelatinous tissues around the cloaca make it difficult to close it in any way. It proved to be a most instructive thing to cause the injection mass to flow into the tubules slowly, and to watch with a lens its course. Injections with lamp-black agar and subsequent digestion with pepsin and hydrochloric acid were unsatisfactory; for the delicacy of the structures made it impossible to isolate complete tubules.

The ordinary methods of histological study were employed. The reconstruction was made by the Born wax-plate method.

2. Tubular System of Wolffian Body in Human Embryos.

In all higher Amniota the first part of the urinogenital apparatus to make its appearance is the Wolffian duct. According to Hensen and v. Spee this tube is derived from the ectoblast; while His and Kowalewsky believe that it has its origin in the middle plate of the mesoblast. Remak, v. Kölliker, Waldeyer, and others trace its development from the lateral plate of the mesoblast. Romiti, Rensen, Dansky, and others find that it springs from the cœlomic epithelium. According to Michalkovics it is at first a solid mass of cells differentiated from the mesoblast. The canal is blind at both ends in the beginning, and becomes lined by an epithelium-like layer.

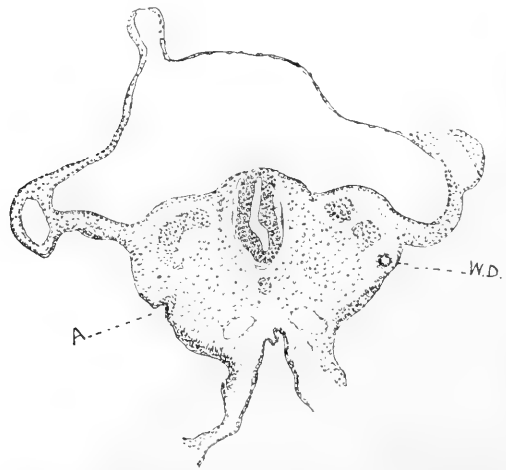

Fig. 1. Transverse section of human embryo CLXIV (3.5 mm.) in length. A, beginning of anterior part of urinary apparatus. W. D., Wolffian duct.

In a human embryo, CLXIV,[1] 3.5 mm. in length, possessing 19 myotomes (probable age 2½ weeks), the Wolffian duct is found in an early stage of its development. It is in close connection with the cœlomic epithelium in many places; and at its anterior extremity is evidently a direct turning in of the lining of the cœlom, Fig. 1 A. The duct consists of a rod of cells ending anteriorly in a depression or groove. This is shown on the left side of Fig. 1; while on the right side the duct is cut more posteriorly and shows already some indication of the formation of a lumen. Examined closely, the organ is found to consist of two parts, an anterior, and a posterior division. The anterior part begins opposite the 6th myotome, and ends opposite the 9th. The posterior part begins opposite the 10th myotome and extends throughout the rest of the body to the level of the last myotome. This is shown diagrammatically in Fig. 2. The anterior part, A., is a simple rod-shaped mass of cells possessing a small lumen at the anterior end opening into the body cavity, as shown in Fig. 1. The posterior part, W. B., extends backward parallel with the last nine myotomes; and at 13 places on its course it is thickened to form rounded masses, from a few of which there project lateral outgrowths. These are the beginnings of the Wolffian tubules, W. T. At this stage there is no indication whatever of glomeruli.

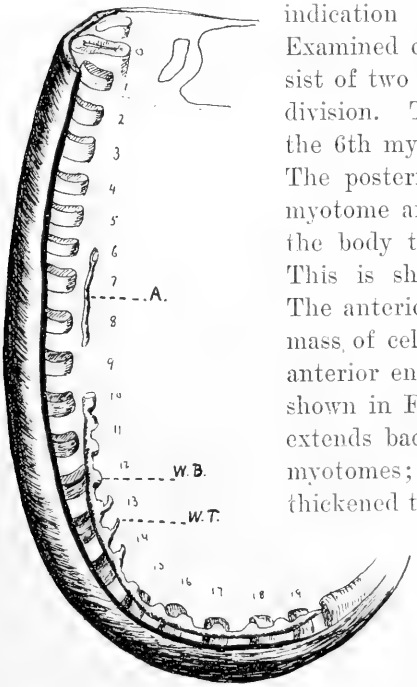

FIG. 2. Diagrammatic reconstruction of urinary apparatus in human embryo CLXIV. A., anterior region of apparatus; W.B., Wolffian body; W.T., tubules. The myotomes are numbered.

The significance of these two parts of the urinary apparatus is not clear. One is tempted to consider the anterior mass of cells as in some way related to the pronephros of lower animals; and the posterior mass, as the developing Wolffian body. The fact that the anterior mass possesses a lumen which opens into the body cavity would seem to support this idea, as this is the case with the pronephric tubules

[1] The Roman numerals refer to embryos in the collection of human embryos belonging to Dr. Mall, in the Anatomical Laboratory of the Johns Hopkins University.

in lower vertebrates. Between the anterior and posterior parts there is a space of five or six sections, each 20 μ thick.

In a somewhat older embryo, LXXX (V. B. 4.5, N. B.[2] 5.; probable age 3 weeks), a slightly more advanced stage in the development of the tubules is noticed. The urinary apparatus begins opposite the 7th myotome as a small duct. This extends for 4 sections, each 20 μ thick, and then ceases. During its course one small, blind, slightly curved, tubule is given off. In the next four sections no trace of this duct can be made out. In the section following, however, another duct starts and is continued to the posterior end of the body. It seems at first probable that this interruption in the duct may be due to a misplacement of four sections in the series; but a close examination shows that this is quite impossible. Furthermore, the tubule which arises from the anterior duct proceeds from its ventral side and curves with its convexity ventralwards; while the tubules of the posterior duct all arise from the dorsal side of the duct with the convexity dorsalwards, as shown in Fig. 3.

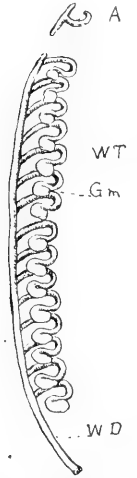

Fig. 3. Diagrammatic reconstruction of urinary apparatus in human embryo. LXXX, (V. B., 4-5, N. B., 5) Lettering as above. Gm, glomerules.

There are no glomeruli opposite the anterior duct; while in the posterior part of Wolffian body proper, there is a glomerulus corresponding with each tubule. The tubules are curved as represented in Fig. 3 and number 15 on one side of the body and 17 on the other. There proved to be 17 glomeruli on one side and 18 on the other. Considering the possibility of error in counting these by following them through a series of sections, it seems that the organ is a fairly symmetrical one; and that there are approximately as many tubules as glomeruli, and an equal number of each on the two sides.

A very similar condition is met with in another embryo, LXXVI (V. B. 4.8, probable age 3 weeks). Here also the urinary apparatus consists of an anterior and a posterior part. Opposite the 7th myotome the duct first appears. At the upper end of the 8th it ceases, and a new duct begins at the lower end of the 8th myotome. The latter duct extends backward to the posterior end of the body cavity. The tubules have a structure similar to that described in the preceding embryo. There is no differentiation into a secretory and a conducting part, Fig. 4. As nearly as can be determined there are 19 tubules in the Wolffian body

[2] V. B. and N. B. refer to the vertex-breech and the neck-breech measurement.

of this embryo. The Malpighian bodies are not fully formed. A crescent-shaped bending of the end of the tubule is present with the concave side thickened, and the opposite side thinned out to a layer of flat cells. A small mass of capillaries is pushed into the concave side of this end structure, as shown in Fig. 4. The tubule is curved like the letter S. The lining epithelium of the Wolffian duct is not different in any essentials from that of the tubules.

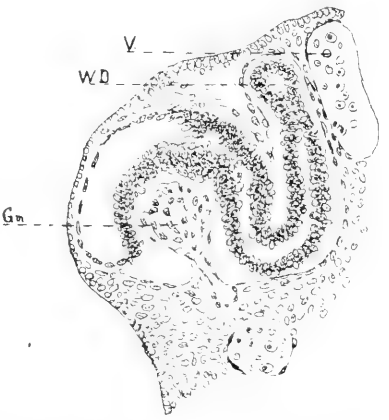

FIG. 4. Transverse section of Wolffian body of human embryo LXXVI, (V. B. 4.8). The Malpighian corpuscle is shown in process of formation.

An older human embryo, II (V. B. 3, N. B. 7, probable age 4 weeks), shows a slight advance on this last stage. In the Wolffian body there are 30 tubules and 30 glomeruli. These correspond throughout with the greatest accuracy. There is no trace of the short anterior duct described in the preceding embryos. The Wolffian tubules are S-shaped, with a slight dilatation near the Malpighian body. Except for this there is no differentiation into a secreting and a conducting region. In an embryo, CLXIII (V. B. 9, N. B. 9, probable age 4½ weeks), this differentiation is well marked. The duct itself is lined by regularly arranged cells. The tubules near the duct possess a small lumen and are lined by small polygonal cells. In the region of the Malpighian body the lumen becomes considerably wider, and the cells lining the tubule are large and rich in protoplasm. This difference, which was first noticed by J. Müller, is seen in a human embryo about 5 weeks old, CIX (V. B. 11, N. B. 10.5). In embryo CXLIV (V. B. 14, N. B. 12, probable age 5½ weeks), the Wolffian body possesses 27 tubules and approximately 25 glomeruli. Figure 5 is a longitudinal section of the Wolffian body taken from a sagittal series of the embryo, showing the relation of the organ to the testis and kidney. The close relation between the Wolffian body and the testis in which tubules are just beginning to develop, must be noted. That these tubules become connected with the Wolffian body tubules through the Malpighian bodies, and that their subsequent connection with the epididymis is thus established will be shown later. In this embryo the Müllerian duct can be seen only as a very short tube extending back-

ward from the peritoneal cavity at the anterior end of the Wolffian body to end blindly posteriorly.

In a somewhat older embryo, CXXVIII (V. B. 20, N. B. 14, probable age 11 weeks), there are distinct signs of retrogression in the Wolffian body. There are 20 tubules on each side, while in younger embryos as many as 30 were observed. The most anterior tubules possess a somewhat widened lumen, and the most posterior show signs of obliteration. Tubules half filled with cells can be made out together with the remains of glomeruli. Into the anterior 8 or 9 Malpighian bodies there is a growth of the testis tubules. The Bowman's capsule is broken through by the testis tubules and a connection thus established between testis and epididymis. This will be described more in detail in pig's embryo, where fresh material allowed of its more exact study.

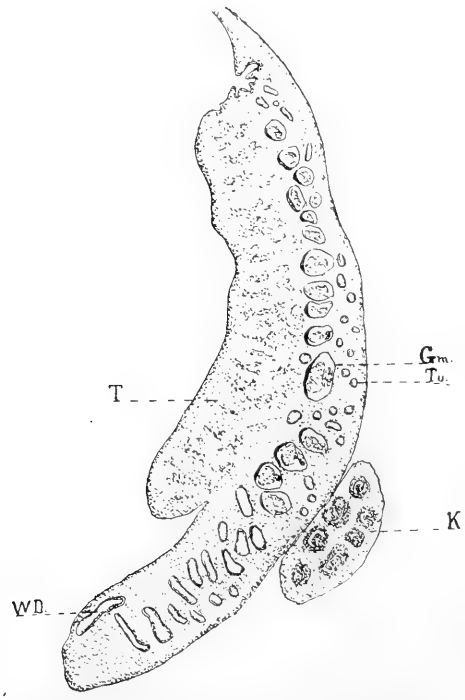

FIG. 5. Longitudinal section of Wolffian body, testis, and kidney, from human embryo CXLIV. (V. B. 14, N. B. 12.) T., testis; Tu., Wolffian tubule; Gm., glomerulus; K., kidney.

In embryo LXXXVI (V. B. 30, N. B. 20), there are 9 tubules and 12 glomeruli in the left Wolffian body. It is evident in this specimen that the degeneration of tubules progresses from the posterior end of the organ forwards. In another embryo, LXXV (V. B. 30, N. B. 20), there can be seen in the posterior half of the organ only vestiges of tubules. The anterior end is somewhat enlarged. The tubules here are considerable coiled, and the Malpighian bodies are in close connection with the tubules of the testis.

From the above notes, a rough idea of the course of development and metamorphosis undergone by the Wolffian body in man can be arrived at. The anterior tubules (pars sexualis) continue to increase in length and complexity to form the head of the epididymis in the male, and the parovarium in the female. The more posterior tubules (pars renalis)

form in the male the paradidymis or organ of Geraldé, and in the female the paroophoron. The Wolffian duct persists in the male as the tail of the epididymis and the vas deferens, and in the female as Gartner's canal.

The Müllerian duct in the female forms the Fallopian tube and uterus. In the male the middle part disappears. The anterior portion gives rise to the hydatids of Morgagni, the posterior to Weber's organ. When the whole tube persists it is called Rathke's duct.

3. Tubular System of Wolffian Body in the Pig.

The youngest pig's embryo I was able to obtain was 8 mm. in length. At this stage the Wolffian body is fairly well formed. In Fig. 6 it is shown in transverse section. It is made up of a tubular and a glomerular part. The glomeruli are situated ventro-medially throughout nearly the whole length of the organ. At the posterior end they cease a short distance anterior to the hindmost Wolffian tubules. They are directly connected with the aorta by a series of arteries which run across in a straight line through the dorso-medial portion of the gland, Fig. 6. The Wolffian duct runs in a slight ridge along the outer ventral border of the gland, and extends from the anterior end of the Wolffian body to the cloaca. In its course it is slightly curved with its concavity towards the median line. From it there proceed at right angles a number of tubules, each of which has a lumen considerably smaller than that of the duct. These have the course represented in Fig. 7. In this embryo there were 51 glomeruli and 42 tubules on the left side; 45 glomeruli and 40 tubules on the right side.

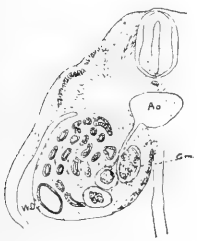

Fig. 6. Transverse section of embryo pig 8 mm. long. W.D., Wolffian duct, Gm., glomerulus, Ao., aorta.

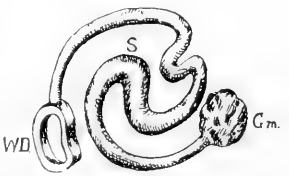

Fig. 7. Diagrammatic reconstruction of Wolffian tubules and duct from pig's embryo 8 mm. long.

The Wolffian body reaches its greatest development when the embryo is about 40 mm. long. From this stage to the time when it reaches a length of 95 mm. the gland remains in about the same condition. After this degeneration begins and changes take place which end in the almost complete disappearance of the organ.

In the Wolffian body, at the height of its development in pigs between 40 and 95 mm. in length, there can be recognized three main surfaces. The ventro-medial and dorso-medial sides are flattened by pressure from

the sexual gland and the kidney respectively. The remaining lateral surface is rounded, as shown in Fig. 13. Three borders may be spoken of: the ventral border, which is caused by the ridge in which the Wolffian duct is situated; the medial border between the ventro- and dorso-medial surfaces, and the more or less rounded dorsal border. The blood-vessels enter and leave the gland at the medial border. The glomeruli are situated beneath the ventro-medial surface.

An attempt was made to determine the course of the tubules by means of injections. Although by this method it was not possible to obtain

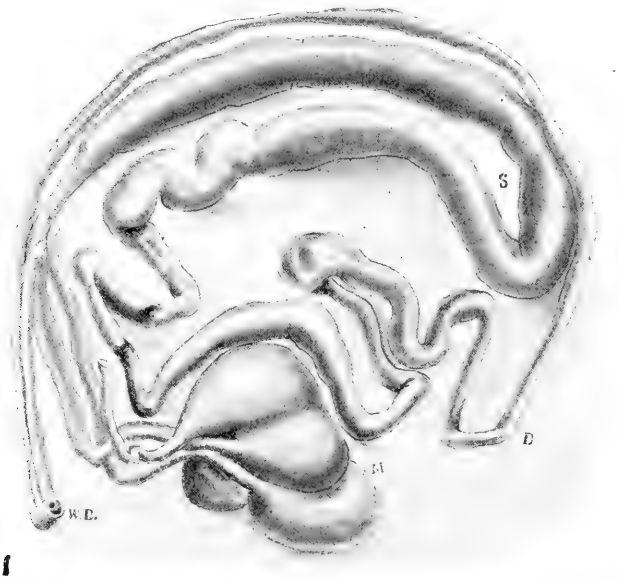

FIG. 8. Wax reconstruction of the Wolffian body of a pig's embryo 80 mm. long. S, secretory portion of tubule; M, Malpighian bodies; D, dorsal border; W. D., Wolffian duct, anterior border.

specimens showing the entire course of any one tubule, some interesting facts were arrived at. By filling the allantois with colored fluid and keeping up a constant pressure on it, the injection could be followed with a lens. The fluid could be seen to run rapidly up the Wolffian duct almost to its anterior extremity. A moment later the fluid entered the tubules, beginning with the most posterior ones. In these it could be followed around the lateral surface of the gland to the dorsal border, where it plunged into the depths. A moment afterwards it entered the capsules of the glomeruli. This same process could be followed until the anterior end of the organ was reached. If pressure enough

was exerted to fill the anterior tubules, the posterior ones became much dilated and overfilled. On the lateral surface the fluid at a certain point could be seen to run in opposite directions in the tubules on the surface, and in those just beneath these, which will be explained in the study of the entire course of the tubules. Partial injections were found to be very instructive. It was observed that some of the tubules branched soon after leaving the Wolffian duct. In examining thick sections of these injected specimens cleared in creosote, the tubules were seen sometimes to branch just before entering the glomeruli. In this way one tubule might be in connection with two or more glomeruli. Evidences of anastomosis and the formation of small networks of tubules were also made out. This was seen particularly in the region of the of the dorsal border.

To gain an exact idea of the course which the tubules take in the gland, a wax reconstruction was made according to the method of Born. This well-known method has been described in detail by Bardeen.[3] Wax plates, 2 mm. thick, and a series of sections cut at 10 μ were used. Every other section was reconstructed and controlled by the intervening ones. The magnification was thus 100 diameters. The model is represented in Fig. 8. The course of the tubule can be made out plainly from its beginning in the Malpighian body to its termination in the Wolffian duct. The Bowman's capsule (to use a term usually employed in describing a similar structure in the permanent kidney) narrows down to a fine tube which runs forwards towards the ventral border. Here it turns and follows the lateral surface of the gland to a short distance from the dorsal border, where it turns abruptly on itself, forming a large loop, and returns to the region of the anterior border. Here it becomes somewhat convoluted and then passes over to the region of the dorsal border, where it is again thrown into convolutions. From the dorsal border it proceeds around on the lateral surface of the gland to empty into the Wolffian duct. Certain differences in the calibre of the tubule are to be noted. The collecting tubule arising in the capsule of Bowman is small, and is lined by cubical epithelium. In the region of the lateral surface it passes into a tube many times larger, lined by large columnar epithelial cells containing granular protoplasm. These cells seem to be secretory in character. This large tube forms a complete loop, as shown in Fig. 8, and passes over in the region of the anterior border into a much smaller, somewhat convoluted segment of the tubule. This in turn runs across the surface of the Malpighian bodies, where it becomes again greater in diameter, to join with another convoluted

[3] Bardeen: Johns Hopkins Hospital Bulletin, April–May–June, 1901.

segment in the region of the dorsal border. This whole middle part of the tubule has a much greater diameter than either the collecting tubule at the glomerulus end or that which empties into the Wolffian duct. The relative size of these various segments is shown in Fig. 8. Special names might be given to the different parts of the tubule, but until their significance is more definitely known this could be of little value. There is, however, a very distinct division into a secretory and a conducting part. In the two convoluted segments, anastomoses sometimes occur. It can readily be seen in examining the course of this tubule how fluid forced into it from the Wolffian duct could be seen on the lateral surface running in opposite directions. In comparing Figs. 7 and 8 the development of the tubule can be roughly traced. In Fig. 7 the large secretory loop S can already be recognized. The greatest increase in length thus takes place in the segment between this loop and the Wolffian duct.

The epithelium lining the large secretory loop and the larger parts of the middle segment of the tubule is represented in Fig. 9, S. T. The cells are large, cylindrical, and somewhat rounded. The protoplasm is granular in the basal half of the cell and quite clear in the other half. The nucleus is oval and situated near the centre of the cell, usually at the edge of the granular half. The epithelium lining the collecting tubules, Fig. 9, C. T., is made up of cubical cells rich in granules throughout. The nuclei are round and stain deeply in hæmatoxylin. The lines of demarcation between these cells are not plainly visible, while in the secretory portions of the tubule each cell can be seen distinctly.

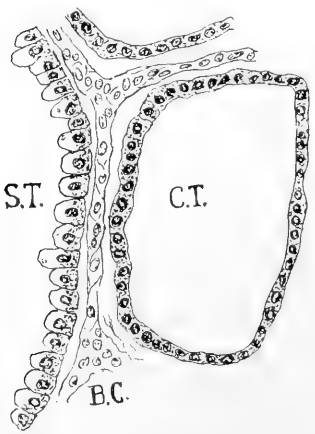

FIG. 9. Section through Wolffian tubules showing secreting tubule (S. T.) and collecting tubules (C. T.). B. C., blood capillary.

Evidences of degeneration can be observed in injecting the Wolffian tubules of pigs 100 mm. in length. At this stage the tubules sometimes inject completely, while in other specimens the fluid runs only a short distance. In the male there is usually left a small uninjected region opposite the testis. The tubules injected anterior to this become the epididymis. At this stage also many of the tubules contain desquamated epithelial cells cast off into the lumen.

In pig's embryos 120 mm. in length, the injection fluid cannot in

any case be forced through the entire length of the tubules. Usually only an occasional tubule near the middle of the gland, and in the male the fine tubules at the extreme anterior end are filled with the colored solution. An injection of the gland in the female at this stage is shown in Fig. 10. It will be noticed that both the Wolffian and Müllerian ducts are injected. The organ is drawn from its lateral surface to show the extent to which the Wolffian tubules have been injected. The tubules are by no means so abundant as in younger embryos. The glomeruli are still present in considerable numbers. The interstitial tissue is relatively greater in amount than in earlier stages. Degenerating tubules in such a gland are shown in section in Fig. 11. In the upper tubule the lumen is seen to be partially filled with epithelial cells, while in the lower tubule the lumen is almost obliterated.

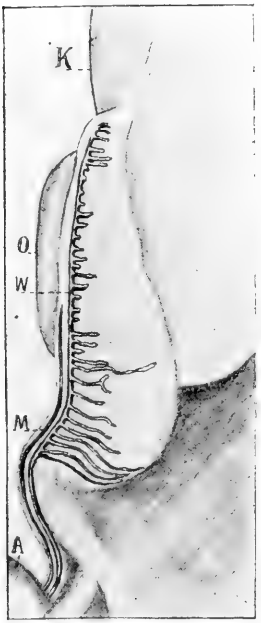

Fig. 10. Injection of Wolffian and Müllerian ducts in a pig's embryo 120 mm. long. O., ovary; M., Müllerian duct; W., Wolffian duct; A., allantois; K., kidney. The organ is viewed from the side.

In embryos 130 mm. long the tubules cannot be injected at all in the posterior (urinary) part of the Wolffian body. In the female the fluid runs up in the Müllerian duct and flows into the body cavity. In the male there is a complete injection of the anterior (sexual) part of the organ, *i. e.*, the epididymis. In pigs 140 mm. long it requires considerable pressure to force fluid into the Wolffian duct. On entering, however, it runs up to the anterior end and flows out as before into the tubules near the head of the testis. In embryos 145 mm. long the injection fluid fills a considerable mass of tubules representing the head of the epididymis. In the female it requires only very little pressure to cause the fluid to flow through the Müllerian duct to the body cavity. Here the Wolffian duct can no longer be injected.

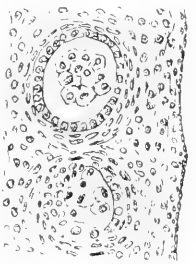

Fig. 11. Section showing obliteration of tubules in a pig's embryo 120 mm. long.

4. Blood-Vessels of Wolffian Body of Embryo Pig.

The *arteries* of the Wolffian body in an embryo 40-80 mm. in length arise from the lower half of the aorta. Their number varies from five to eight. They run in a slant direction posteriorly across the lower part of the kidney and enter the Wolffian body at its medial border. Entering the organ here they break up into branches which proceed to the glomeruli. The position of the latter in the organ has been described. It is shown again in Fig 12. Each arterial branch

Fig. 12. Thick transverse section of Wolffian body of a pig's embryo 45 mm. long. The blood vessels are injected. The arteries, veins and glomeruli can readily be distinguished.

Fig. 13. Ventral aspect of Wolffian body of a pig's embryo 45 mm. long, showing the surface veins and the arteries entering the organ.

Fig. 14. Cross section of Wolffian body, kidney, and sexual gland, showing the relation of their veins.

Fig. 15. Three tubules from a thick section of the Wolffian body of embryo pig 45 mm. long, showing the capillary plexuses in the walls.

may supply one or more glomeruli; or, on the other hand, one glomerulus may receive several branches from one artery. The afferent arterial branches break up to form the large plexus of capillaries making up each glomerulus. No definite arrangement of these capillaries can be made out. The glomeruli are many times as large as those of the permanent kidney. From each glomerulus there arise two or more efferent arteries. These usually proceed from the side opposite the entry of the afferent vessels. As many as five of these are often seen in a thick section. They run out radially from the glomeruli and form networks of capillaries around the Wolffian tubules. From these the veins arise, as shown in Fig. 12.

The *veins* of the Wolffian body arising from the capillary networks around the tubules are represented in Fig. 12. They gather together in two directions. A large number join to form veins which proceed towards the periphery of the organ, while the rest enter large veins which leave the Wolffian body by the hilus where the arteries entered. The surface veins are large branching vessels which run somewhat parallel with the tubules and divide the whole organ roughly into lobes. Their course on the ventral aspect of the organ is shown in Fig. 13. Arising from between the tubules they course over the surface and pass under the Wolffian duct. Figs. 12 and 13. On the ventro-medial surface of the organ they join together to form three or four large trunks, which enter a common vein at the medial border. From the dorsal region veins present a somewhat similar picture. They usually gather together into four large vessels, two of which drain the middle third of the gland, while the other two drain the anterior and posterior thirds. Running along the dorso-medial surface, these venous trunks join with the veins from the ventro-medial surface and enter the inferior vena cava. In addition to these surface veins, there is a series of central veins which leave the Wolffian body at the medial border. One of these veins is shown in Fig. 12. It is made up of the junction of several small branches derived from the capillary plexuses around the tubules. Passing down on the dorsal side of the glomeruli, it joins with the superficial veins to enter the common trunks.

Fig. 14 shows the relations of these three series of veins and their relation to the veins of the testis and kidney.

It is necessary to note particularly the disposition of the efferent arteries as shown in Fig. 12. As mentioned before, they pass out from the glomeruli in a radial direction. Each artery in this way occupies a territory of its own, and from all sides its small branches form capillary networks which collect to form the veins. This is a repetition of what has been noted in many organs, namely, the formation of blood vascular units with an artery in the centre of each and veins at the periphery. In a transverse section, such is as represented in Fig. 12, six or seven units can be observed. The arterial end of each capillary plexus can be easily distinguished from the venous end by a difference in structure just as the arteries and veins can without difficulty be recognized in a single injection of the Wolffian body. The venous end of this network is shown in Fig. 15. This figure is intended to represent a thick section of three tubules, the walls of which are seen obliquely. A fine plexus of irregular venous capillaries covers the walls and gives evidence of the remarkably rich blood supply of the organ.

5. Relation of the Tubular Systems of the Testis and Wolffian Body.

An intimate relation between the tubular systems of these two organs was spoken of in describing the human organs. In pigs' embryos the same relation can be made out with much greater distinctness, owing probably to the possibility of obtaining fresh material. In the pig the sexual gland is from the first very closely connected with the Wolffian body. It develops on the ventro-medial surface of the anterior part of the organ apparently from the cells covering this surface. Later on tubules develop in the testis, and the large characteristic sexual cells in the ovary. The exact method of development of these glands does not come within the scope of the present paper. It is sufficient to note that in a pig's embryo 95 mm. long the testis tubules

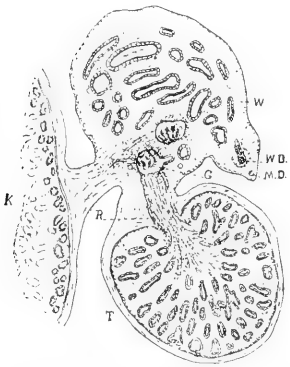

Fig. 16. Transverse section of Wolffian body, testis and part of the kidney of embryo pig 95 mm. long. R., tubules from testis breaking into the Malpighian body; T., testis; K., kidney; W., Wolffian body, W. D., Wolffian duct; M. D., Müllerian duct.

are well developed, showing well-marked walls and a distinct lumen. In the centre of the gland and towards the hilus the tubules become very narrow and enter a mass of extremely fine tubules which run together to the hilus of the testis. These tubules are so small that it is difficult to observe a lumen in many of them. As shown in Fig. 16, the mass of tubules leaves the testis and passes directly over into the Wolffian body through the neck of tissue which joins the two organs.

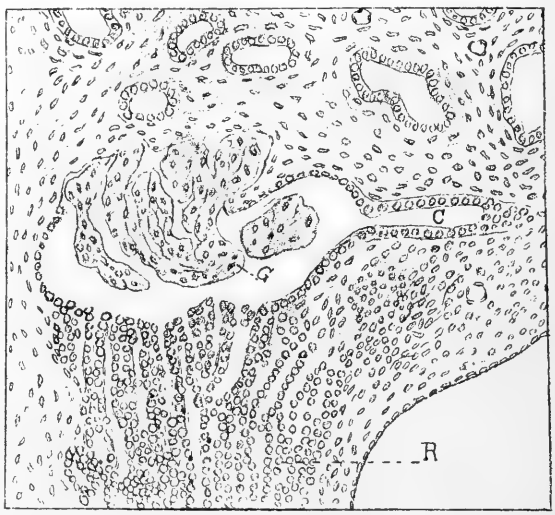

Fig. 17. A portion of the preceding section with higher magnification. R., tubules from testis; G., glomerulus; C., cavity of tubule and Bowman's capsule. The entrance of the testis tubules into this cavity can plainly be seen.

Here they come into contact with the Malpighian corpuscles, with the walls of which they seem to fuse, so that the tubules come to communicate with the cavities contained by Bowman's capsules. This is shown in Figs. 16 and 17. It will readily be seen that a communication is thus established between the testis tubules and the Wolffian duct through the Malpighian bodies and tubules of the Wolffian body. The testis tubules break into only the anterior ten or twelve Malpighian bodies, so that the anterior tubules (10 or 12) are the only ones in connection with the testis. From these tubules the head of the epididymis must be formed. From the Wolffian duct the rest of the epididymis and the vas defferens arise. This mode of secondary communication between the testis and the Wolffian tubules is not unknown in the lower classes of vertebrates.

ON THE VITELLINE VEIN OF THE CAT.

BY

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WITH 8 TEXT FIGURES.

Although the vitelline (omphalo-mesaraic) artery is synonymous with the superior mesenteric, yet the vitelline vein is not identical with the superior mesenteric vein. The artery, at all stages of the animal's life, is found in the mesentery of the jejunum and ileum. Fig. 1 is a transverse section of a cat embryo of 7.6 mm. The section is published not because it shows anything which is unknown, but because it happens to be a fortunate section and demonstrates at a glance the course and relations of this vessel. The artery is seen in the mesentery of the intestine; it crosses the ileum, and divides at its termination to surround the yolk sac.

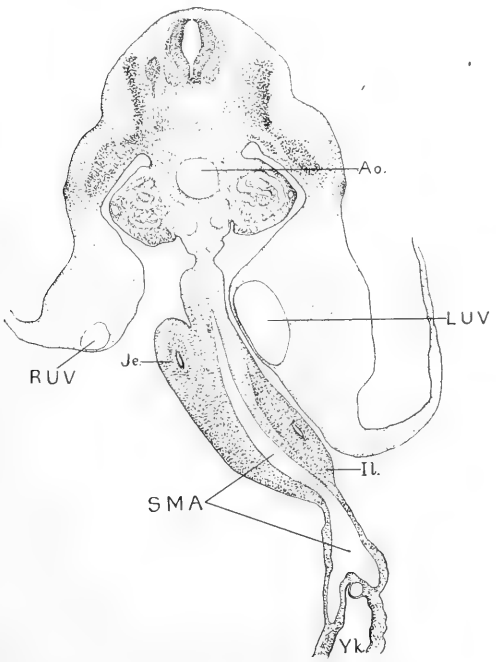

FIG. 1. Transverse section of a cat embryo 7.6 mm.

After the obliteration of this sac, that portion of the artery ventrad to the intestine, lies in the tissue of the umbilical cord, and consequently, fixes or anchors the loop of ileum which it crosses in the cavity of the umbilical cord, until all other portions of the intestine have entered the cœlom proper. The artery then elongates, and thus finally allows

the loop of ileum to enter the cœlom. This process was described by me in a paper two years ago.[1] At birth, and even for a few days after birth, this free, elongated artery may be easily identified, and, curiously enough, in the cat, remains pervious up to the fourth or fifth day. It represents the terminal branch of the superior mesenteric, and in the embryo is undoubtedly its largest branch.

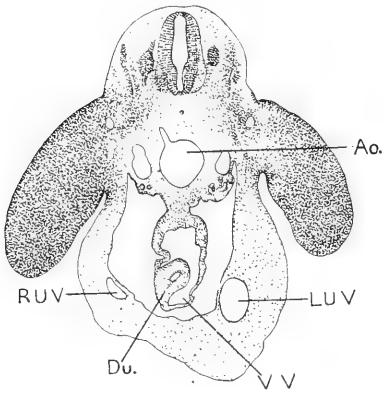

FIG. 2. Cat embryo of 6.2 mm. Harvard Embryological Collection. Transverse Series 380. Section 272.

Our knowledge of the liver veins is largely due to the admirable researches of Hochstetter, as well as to others who have written on this subject, not to mention what is to be found in the many text-books on anatomy. As far as I know, Hochstetter, as well as the others, treats of the course and relations of the vitelline veins after they have reached the duodenum, rather than of the first part of their course, namely, from the yolk sac to the duodenum. I have found no literature relating to this division of the subject. Both the right and left vitelline veins are to be seen in a cat embryo of 3 mm. They extend from the yolk sac, within the duodenal walls, encircle its cavity, and finally terminate in the heart. Soon after this date the right vein, below the liver, is obliterated, and the

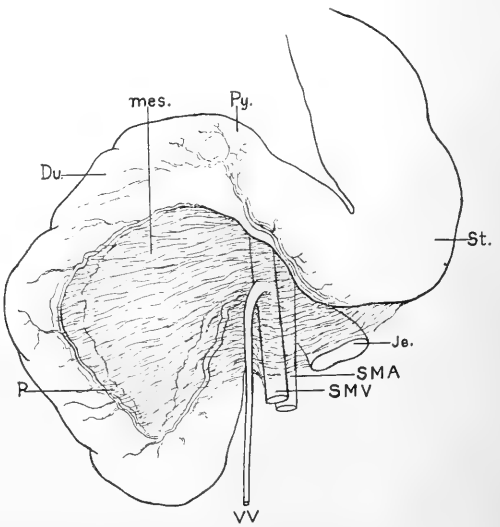

FIG. 3. A drawing of the stomach and duodenum of a cat at birth to show the free vitelline vein joining the superior mesenteric vein.

[1] Additional Observations on the Morphology of the Digestive Tract of the Cat. The Journal of the Boston Society of Medical Sciences. Vol. IV., p. 205. April 1900.

left alone remains. Notice once more the course which the vein takes from the yolk sac to the liver, and especially its relation to the duodenum. It lies at first ventrad to the duodenum; it next lies laterad to it, then dorsad, and, lastly, cephalad where it reaches the liver.

Fig. 2 is a transverse section of a 6.2 mm. embryo cat. It shows at this stage the relation of the vein to the duodenum. The vein lies within its walls and encircles it.

Fig. 3 is a drawing of the duodenum and its mesentery in a cat at birth. The vitelline vein rests upon the ventral surface of the duodenal mesentery, and is seen to perforate it just below, or caudad to the pylorus, in order to join the superior mesenteric vein. It is obvious that the relation of the duodenum to the vitelline vein in a 6.2 mm. embryo is absolutely different from that in a cat at birth. (Figs. 2 and 3.)

The following five figures represent transverse sections of cat embryos at various stages of development. They were all drawn on the same scale by means of a camera lucida.

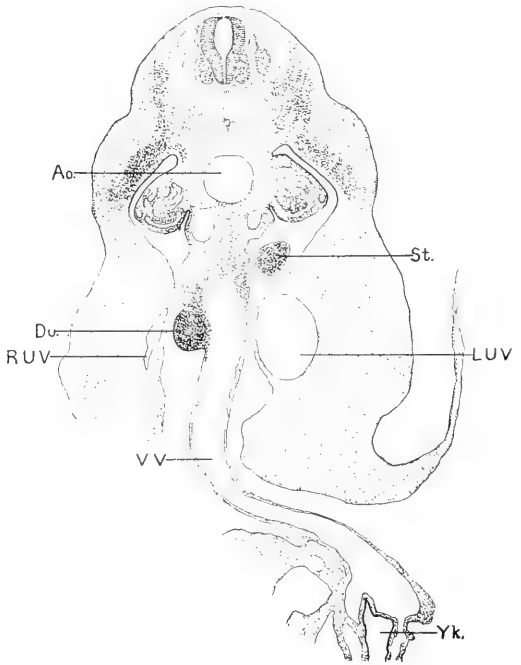

Fig. 4. Transverse section of a 7.6 mm. embryo.

In all the drawings the relation of the vitelline veins to the aorta remains constant, but the relation of the duodenum to the aorta gradually changes. The vein remains fixed, but the duodenum, by means of an extensive growth of its mesentery, migrates to the right, and thus produces the peculiar relation of the vein to the duodenum which is seen in a cat at birth. Besides the stages figured, I have examined several intermediate stages which fully confirm the history of the development as given below. The growth is gradual. Now let us examine it step by step.

As has already been pointed out in Fig. 2, the vitelline vein lies on

three sides of the duodenum. The relation has changed in Fig. 4, which is a section of a 7.6 mm. embryo. Here the vein is purely laterad to the intestine.

There is no very great change in the relation of the duodenum to the vitelline vein in an embryo of 12 mm. (Fig. 5) as compared with the last stage (Fig. 4), except that the mesenchymal tissue which forms

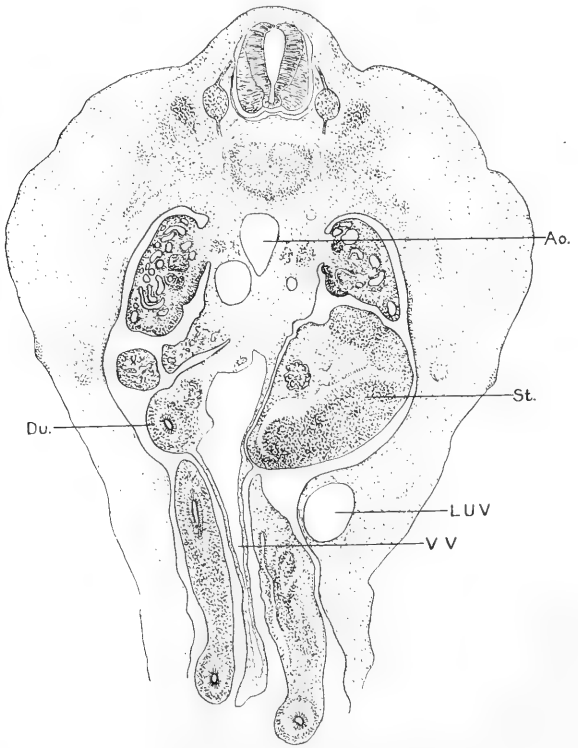

Fig. 5. Embryo of 12 mm. Harvard Embryological Collection. Transverse series 399. Section 460.

the walls of the gut is much thickened, its lumen is farther from the vein, and the embryo is more developed in every respect.

In the section of a 15 mm. embryo (Fig. 6) a pronounced change has taken place. The vein may now be described as being in relation to the mesentery of the duodenum, rather than in relation with the walls of the intestine itself.

Figs. 7 and 8 are sections of the duodenum, its mesentery, and the vein, in embryos of 23.1 and 39 mm., respectively. The same extensive

growth has continued, and now at 39 mm. the gut is found at an appreciable distance from the vein. The continued growth of the duodenum to the right, together with the immobility of the vein, fully accounts for the change in relation to the duodenum and vitelline vein as seen in the youngest embryo, and in the cat at birth.

We have already seen (Fig. 1) the relations of the vitelline artery

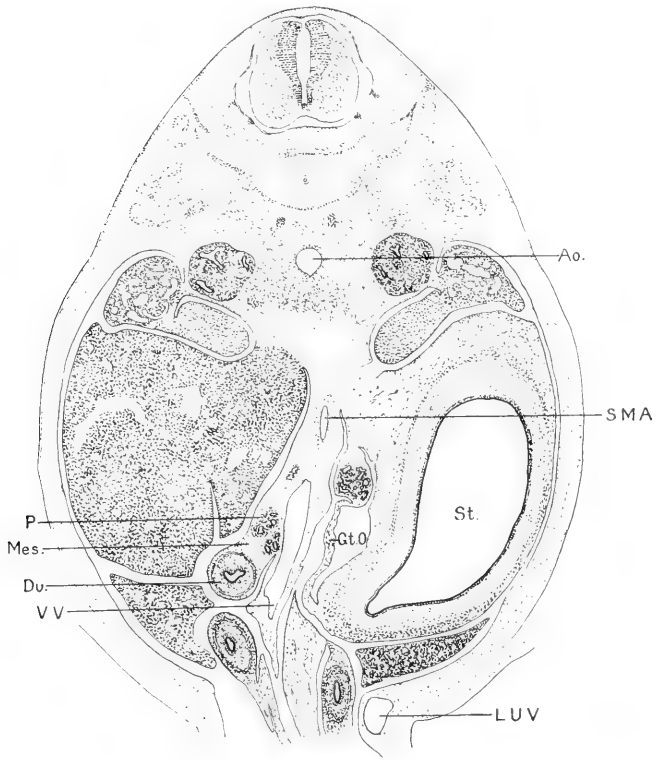

Fig. 6. Embryo of 15 mm. Harvard Embryological Collection. Transverse series 436. Section 527.

throughout its entire extent. Fig. 4 is taken from the same series, the twelfth section cephalad to it. It shows remarkably clearly the course of the vein from the yolk sac to the duodenum. The vein is seen to be unconnected with any mesentery. It lies absolutely isolated and alone until it reaches the mesentery of the duodenum.

One can easily identify, either in this section, or in one made at right angles to the intestine, its endothelial lining, which is surrounded by a

variable amount of mesenchymal tissue, and covered by the mesothelium. It is obvious that this vessel cannot be a synonym for the superior mesenteric vein, since that vein lies with the artery of the same name in the mesentery of the small intestine. I know nothing of the development of the superior mesenteric vein, but if one injects the portal system of a cat at birth with very thin Teichmann's mass, the pervious vitelline vein can be seen entering the mesentery of the

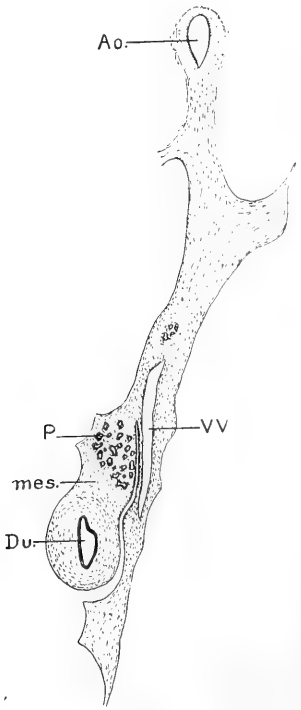

Fig. 7.

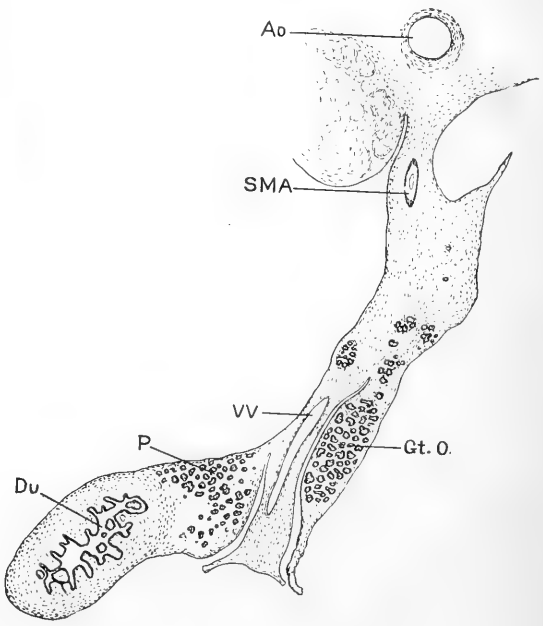

Fig. 8.

Fig. 7. Embryo of 23.1 mm. Harvard Embryological Collection. Transverse series 466. Section 1079.

Fig. 8. Embryo of 39 mm. Harvard Embryological Collection. Transverse series 361. Section. 643.

duodenum, to join the superior mesenteric vein just previous to the union of that vein with the splenic. In other words, the vitelline vein is no more a branch of the superior mesenteric than is the splenic vein. It does not lie in the mesentery of the jejunum and ileum, neither does it receive blood from the intestines. Its object seems to be to return the blood from the yolk sac to the liver, and in its course it joins the

superior mesenteric vein, to aid, together with other veins, in the formation of the portal system. It is certainly most difficult to understand why both the artery and the vein should be pervious after birth, and especially such a very long time after the obliteration of the yolk sac.

One other point in regard to the vitelline vein. It seems to reach its maximum development in the embryo of 12 mm. (Fig. 5) and then to slowly atrophy. We have already seen that it is pervious for a few days after birth, so it is unlikely that there can be any great change in its size after the embryo has reached a length of 39 mm.

To recapitulate: The vitelline vessels remain pervious for a few days after birth.

As the result of an extensive growth of the duodenum to the right, the vitelline vein changes its position from the wall of the duodenum to the duodenal mesentery.

At no period is the vein found in the mesentery of the jejunum and ileum, but in all stages of development the vein is free from mesenteries in its course from the yolk sac to the wall of the duodenum, or to the duodenal mesentery.

The vitelline vein unites with the superior mesenteric vein to aid in the formation of the portal system.

ABBREVIATIONS.

Ao. Aorta.
Du. Duodenum.
Gt. O. Great omentum.
Il. Ileum.
Je. Jejunum.
L. U. V. Left umbilical vein.
Mes. Mesentery of duodenum.
P. Pancreas.
Py. Pylorus.
R. U. V. Right umbilical vein.
S. M. A. Superior mesenteric artery.
S. M. V. Superior mesenteric vein.
St. Stomach.
V. V. Vitelline vein.
Yk. Yolk sac.

THE DUCTS OF THE HUMAN SUBMAXILLARY GLAND.

BY

JOSEPH MARSHALL FLINT, M. D.

From the Hull Anatomical Laboratory, University of Chicago; and the Hearst Anatomical Laboratory, University of California.

WITH 9 TEXT FIGURES.

The ducts of the salivary glands have a peculiar interest because they represent the paths of development followed by the more highly organized secretory portions of the organ. Excepting possibly certain parts of the intralobular system comparatively little work has been done on the ducts of the human submaxillary gland. Owing to the important embryological relations of the ducts and the interest associated with their functions of providing a channel for the secretion, accurate information concerning their course and structure should be obtained. With this end in view, therefore, the following work was undertaken.

METHODS.

Studies of the gross anatomy of the ducts and the gland were carried on in the dissecting rooms during the regular work of a class in systematic anatomy. The material was embalmed with a bichloride, glycerine and alcohol fluid and injected with red lead and starch. On the whole the cadavers were in very good condition so that the relations and structure of the tissues under investigation and those about them were very well preserved. Entirely outside of the value of the study itself, the pedagogical effect of demonstrating to a working class of students some of the simpler research methods is not to be underestimated. We are indebted to Schwalbe, Cunningham and Mall for the extensive use of dissecting-room material for the purposes of research.[1] The material for the corrosions on which this study of the ducts is largely based was likewise obtained from the cadavers. The submaxillary gland was carefully dissected from its bed with a portion of the D. submaxillaris and injected. Corrosions of the ducts can be easily obtained with the ordinary celloidin carriers, colored with chrome

[1] Bardeen: Bulletin of the Johns Hopkins Hospital, Vol. xii, 1901.

yellow, cinnabar, or Prussian blue. Prussian blue is the best pigment for this purpose as its dark color renders the smallest ramifications visible and its fine granulation often allows the mass to pass easily through the intralobular ducts into the alveoli themselves. When it is desired to inject only the sublobular or lobular ducts chrome yellow or cinnabar should be used, for such masses do not, as a rule, pass beyond these structures into the finer ducts. Celluloid colored by victoria blue is a good mass, its advantage lying in the fact that it can be kept in the air as a dry preparation without shrinkage and does not have to be preserved in glycerine like the celloidin injections. For these corrosions commercial celluloid dissolved in acetone can be used or the celluloid may be made by adding camphor and acetone to celloidin. Apparently the granular pigments do not give such good results with the celluloid mass for the corrosion is liable to crumble after the surrounding tissue has been destroyed. Both the celluloid and celloidin can be freed from the glandular tissue surrounding them by the pepsin hydrochloric digestion fluid or a more rapid destruction of the gland is easily effected by immersing the injected organ in commercial hydrochloric acid. Inasmuch as the ducts are small, the acid does not make the preparations too brittle to be handled. All of our injections have been prepared in this way rather than by the use of the more tedious pepsin method. The stereoscopic microscope proved to be of the greatest service in the study of the corrosions. By its use we can follow, owing to its deep field, the course of the finer branches accurately in three dimensions and get much sharper pictures of the relations of these structures than by the old flat field microscope.

By far the best way of showing ducts in relation to the frame-work of the glands is by a method devised by the writer[2] while working in the laboratory of Prof. Spalteholz in the Anatomical Institute of Leipzig. Small blocks of tissues are hardened in the graded alcohols, bichloride or Van Gehuchten's fluid, dehydrated and then repeatedly extracted with the (Soxhlet) apparatus and digested until all of the glandular elements are dissolved and nothing but the frame-work remains. Up to this point this is the method of piece digestion devised by Spalteholz[3] for the demonstration of connective tissue in sections. After the digestion is complete, the digested frame-work of the organ is then cleared in glycerine, creosote or xylol and is then ready for preliminary study. This block of tissue, owing to the fixation and hardening, retains

[2] Flint: Johns Hopkins Hospital Bulletin, Feb., 1902.
[3] Spalteholz: Arch. f. Anat. u. Phys., Suppl. Bd., 1897.

perfectly the form and relations of the original tissue. It is the delicate opaque skeleton of the original tissue formed by the connective tissue frame-work, and when viewed through the stereoscopic microscope, shows in three dimensions all of the normal relations of the frame-work to the original structures of the organ. Owing to the high diffraction of the fibrils many of the finest details of structure are brought out, as for example, the basement membranes of the alveoli, ducts, vessels, perilobular membranes, etc. When pieces of the submaxillary gland are digested and cleared in this way, the ducts and their accompanying vessels are shown beautifully, both in the interlobular spaces and as they enter the lobule and ramify in its substance.

After careful drawings have been made of these thick preparations in glycerine, they can be utilized for further study with the finer methods according to the original procedure of Spalteholz. When embedded in paraffin and cut in thin sections, they can be stained on the slide with iron hæmatoxylin. Numerous variations in the stains are, of course, possible although iron hæmatoxylin and aniline blue give by far the sharpest pictures. Beautiful specimens can be obtained by using celloidin as an embedding medium and cutting thick sections which are stained in an eight per cent solution of acid fuchsin. They are then washed rapidly in distilled water and the graded alcohols until the celloidin is decolorized, and finally cleared in creosote and mounted. These preparations show the fibrils distinctly for naturally the staining adds greatly to the clearness of the picture, but, at the same time, it is necessary to sacrifice some of the depth as the stained sections cannot be cut over a certain thickness, depending partially on the nature of the tissue and partly on the density of the meshwork.

For the study of the ducts in sections most of the ordinary procedures were employed. Several proved especially useful for this purpose, among which was the method of slide digestion perfected by Spalteholz and his pupils.[4] This consists, briefly, in mounting an alternate series of paraffin sections and digesting one with pancreatin, while the other is stained as a control. To complete this comparison the writer treated a third section by Weigert's elastic tissue method counterstained with picric acid, so as to have, side by side, successive sections prepared by three different methods instead of two. Hensen's modification of the Van Gieson stain[5] proved of value in the study of the connective tissue

[4] Spalteholz: loc. cit. Hoehl: Arch. f. Anat. u. Phys., Anat. Abtlg., 1897. Clark: Ibid., 1898.

[5] Hensen: Anat. Anzeiger., Bd. xv.

in sections, especially in the comparison with digested sections. Many other methods were used and modified as the exigencies of the research required.

Gross Relations.

The Ductus submaxillaris joins the submaxillary gland with the Caruncula sublingualis. Its length varies between four and five cm., its diameter is between two and three mm. The duct first becomes visible as it emerges from the hilus of the gland which is situated usually near the central portion of the medial surface. From the hilus it runs downwards, inwards, and forwards, upon the external surface of the M. hypoglossus running between it and the M. mylohyoideus. After passing the M. hyoglossus it passes in its course between the Glandula sublingualis, the M. genioglossus and M. lingualis inferior. As it runs by the medial surface of the sublingual gland it is usually in intimate connection with the N. lingualis and A. sublingualis. It then terminates in the Caruncula sublingualis which opens into the mouth just at one side of the Frenulum linguae. The walls of the duct are rather thin when the diameter of its lumen is taken into consideration, but it is well provided with elastic and fibrous tunics as well as a few smooth muscle fibres. The contrast in the thickness of the walls of the D. submaxillaris and D. parotideus is at first sight rather surprising, especially as the former carries the thick viscid secretion of the submaxillary gland while the latter forms the channel through which the thin serous product of the parotid is poured into the mouth. When one considers, however, the fact that the duct of the parotid is relatively exposed as it lies covered simply by skin and fascia, this is not so surprising, for the submaxillary duct is well protected and sheltered by the numerous firmer structures forming its environment.

The Gl. submaxillaris lies in the Regio submaxillaris, adapting its form apparently to the shape of the space in which it lies. It is irregularly prismatic or triangular in shape with its large axis directed dorsoventrally, slightly downward and inward so that it lies parallel to the axis of the ramus of the mandible. Below it is covered by the cervical fascia and M. platysma. The V. facialis communis and sometimes the A. maxillaris externa passes over the inferior surface of the gland. Medialwards the Gl. submaxillaris rests upon the M. mylohyoideus, M. stylohyoideus, and M. hyoglossus, while lateralwards the ramus of the mandible forms its chief boundary. On the internal surface is the hilus where the D. submaxillaris leaves the gland. Often there is a posterior prolongation of the organ but this is usually poorly marked. A

small lobe or prolongation, however, is usually observed passing beneath the M. mylohyoideus with the D. submaxillaris in rather intimate association with it. This portion may be completely free from the major part of the gland forming an aberrant lobe, the duct of which joins the D. submaxillaris at a point somewhat below the hilus.

In taking up the description of the course of the secretory channels within the organ it is perhaps best for the sake of clearness to begin with the main duct and then proceed through its complex ramifications to the alveoli, although this course is opposite that taken by the secretion. On the other hand from an embryological point of view, it is, of course, obvious that in adopting this method of description we follow the path taken by the gland in its development. In discussing the course of the ducts it will be necessary to refer, from time to time, to certain facts concerning their development, accordingly, at the outset, it may be well to recapitulate briefly certain details of the organogenesis of the submaxillary which are to form the substance of a later communciation. The first *anlage* consists of a spur from the epithelium of the mouth which marks the beginning of the duct. This *anlage* is a solid cylindrical column of cells which grows and finally begins to branch. The branching portion becomes encapsulated and indicates the primitive form of the organ as we know it in adult life. At this stage it is composed of a blastema of branching nucleated cells in which the growing ducts are embedded. The growth at this stage is chiefly apical and the branches of the simple little tree which later is transformed into the major ducts of the gland terminate in little buds or swellings that form the growing points at the apices. As the gland develops these simple cell columns divide and ramify and become more complex until, after giving rise to the ducts of the first order, interlobular, lobular and intercalary ducts, they produce finally the alveoli and secreting elements of the gland. In their growth, the ducts and their accompanying vessels are surrounded by strands of connective tissue which form later the interlobular spaces. At the time when the ramification has proceeded to a certain point, the growing ends become surrounded by a fine capsule or membrane which marks the initial formation of the lobule and its membrana limitans. This membrane is attached to the growing duct at the future site of the lobular hilus and forms the one firm point of attachment of the lobule. Within this membrane, the intralobular ducts and alveoli are developed. At first the lobules are comparatively free but later become, as they increase in size, closely packed together, forming the irregular polygonal shapes observed in adult life. It is in this way that the limiting membranes of

adjacent lobules are pressed in close apposition, and yet, as a general rule, the attachment between them consists simply of a few fine fibrils of reticulum (Fig. 8). In its early stages, the organ in pigs, as a whole, is regularly symmetrical and the future ducts, marked by the growing columns of cells, branch with great regularity, the larger divisions alternately passing first to one side and then to the other of the gland. They can be followed with some distinctness in ordinary sections but in injected specimens better results are obtained when the gland is divided and viewed with the stereoscopic microscope which shows these relations in three dimensions. In the simplest forms the blood-vessels form a fine plexus about these growing columns of cells and, as they develop and ramify, the arteries and vessels supplying them follow the same line of growth so that we have an artery and veins developing with each branch of the duct.

It has been shown[6] that the intrinsic vessels of an organ indicate in general the paths along which the different parts of that organ have developed, a principle which is expressed in the following paraphrase of a well-known scientific aphorism, viz.: the angiology of an organ in a measure recapitulates its ontogeny. In the case of the submaxillary this principle obtains and the blood-vessels of the organ represent the lines of its development. Therefore, in injected preparations of the developing gland we can follow accurately the course of the ducts from the vessels that always accompany them. Were it essential, this relation of the blood-vessels to the ducts would afford another proof that the ducts themselves also form a record of the development of the gland. It follows, therefore, that the youngest parts of the organ are the terminals of the ducts or alveoli while the oldest portion is the main duct itself. It is also apparent that this relationship of the ducts and vessels gives rise to the invariable conditions observed in the interlobular spaces where an artery and its venæ comites accompany every duct. So constant and regular is this condition that these vessels may be justly termed the vasa comites of the ducts. In embryo pigs the Gl. submaxillaris in gross appearance is a small opalescent organ lying near the angle of the mandible covered by the developing platysma and fascia. At this stage it is not situated in a fossa, nor is it jammed up beneath the mandible. From the earliest period that it can be distinctly seen with the naked eye it is reniform in shape and perfectly symmetrical;

[6] Flint: Monograph on the Adrenal. Contributions to the Science of Medicine, dedicated to Dr. William H. Welch by his Pupils. Johns Hopkins Hospital Reports, Vol. ix, Baltimore, 1900.

the vessels and ducts enter the gland at the hilus. At this stage in its development the organ is encapsulated.

Before the duct of the human gland penetrates the hilus of the organ, it is often joined by a small duct, either from the aberrant lobes which so frequently occur or from the anterior prolongation of the

FIG. 1.—*Celloidin corrosion of the ducts of the human submaxillary gland.* Magnified 4 diameters. The Ductus submaxillaris is shown as the main trunk in this tree, giving off the primary branches just within the boundaries of the gland which are here roughly marked out by the terminal twigs. The secondary divisions are the interlobular ducts. These radiate from the central to the peripheral portion of the gland. The secondary branches of the interlobular ducts form the sublobular system. These divide once or twice and at this point the injection mass has usually stopped, although in a few places it has penetrated into the lobular ducts. The interlobular ducts may divide into one or two larger branches before exhausting themselves in the sublobular system. *A*, Ductus submaxillaris; *B*, primary ducts; *C*, interlobular ducts; *D*, sublobular ducts.

gland which extends with the duct between the M. hyoglossus and mylohyoid. The corrosions by means of which the ducts are studied look remarkably like miniature representations of certain species of trees,

especially the California live oak. That this parallel is not fanciful can be shown by a glance at a corroded preparation of the human submaxillary, where Wharton's duct represents the trunk, the interlobular ducts the branches, the intralobular ducts the twigs, and the alveolar ampullæ the foliage. When the injections are incomplete (Fig. 1) they look like the naked limbs of the oak, but if the mass has passed into the alveoli the corrosion resembles that tree in full foliage. In the corrosion preparations the form of the gland is preserved by the tree as a whole, and these naturally vary in the same wide limits noted in the gross relations of the organ. When aberrant lobes or prolongations are present their ducts usually look like branches arising from the trunk of a tree some distance below the usual branching zone. The size of these portions varies a good deal, but in general they may be said to correspond to that part of the gland which is drained by a duct of the first order.

The submaxillary duct and its ramifications may be classified in the following general scheme, each subdivision representing one of its main divisions, which has, in general, fairly definite relations to the glandular units:

1. Ductus submaxillaris.
2. Primary ducts.
3. Interlobular ducts.
4. Sublobular ducts.
5. Lobular ducts.
6. Intralobular ducts. (Salivary tubes of Pflüger.)
7. Intercalary ducts.
8. Alveolar ampullæ.

At the hilus there is a considerable amount of connective tissue through which the duct penetrates as it enters the gland. In most human submaxillaries the duct divides shortly after entering the hilus; in pigs, apparently, it always does. It is not uncommon, however, to observe in human glands instances where the main duct preserves its identity, penetrating directly through the substance of the gland and exhausting itself by manifold lateral branching instead of dividing into several chief primary divisions just after entering the hilus.

It is somewhat difficult to obtain the diameter of the duct between the hilus and papilla in corroded specimens owing to the nature of the material used since we employed for this purpose the glands removed from bodies in the dissecting room. The walls of the ducts had lost their tonicity and this, together with shrinkage of the celloidin

during the digestion, rendered the corrosions unreliable as a means of determining accurately the caliber of the ducts. These data are naturally best obtained by direct measurements of the distended duct in fresh subjects. In a general way, however, the relations in size are well preserved, although one would hesitate to apply methods of accurate mensuration to them. Of course the ducts in all parts of the gland are under the same general conditions so that the effect of shrinkage in one part would be about commensurate with that in another. And even while we can assume that this method gives a general idea of the relative size of these structures, under no circumstances, however, would we be justified in drawing conclusions from material of this nature as to their exact caliber in life.

In general the method of branching appears to be dichotomous, although often unequally so. The diameter of the two branches after a division is usually unequal, a fact which is especially true of the larger divisions. The rule of dichotomy holds nevertheless throughout the entire secretory system, both intra- and extralobular, with the single exception of the intercalary ducts where three or even four ducts are often given off at a single node. The ultimate alveolar ampullæ, likewise, violate this law since three, four, or five of them always terminate the secretory system (Fig. 3).

The commonest distribution of the ducts is represented in Fig. 1, where the primary branches or ducts of the first order spread out irregularly from a short and twisted trunk radiating in various directions from the hilus. Since the Glandula submaxillaris is about three times as long as it is thick, the branching must be less in the plane of lesser than in the one of greater dimension. Except at the hilus the ducts run, in general, as far away from the capsule as the anatomical conditions which require the drainage of the entire organ will permit. In the human gland the primary ducts do not pass alternately to one and then the other side of the organ, as they do in embryo pigs, but arise rather irregularly from the main trunk. They correspond, however, to the primary divisions of the ducts in embryo pigs and if they had preserved the same regularity of distribution observed in the embryo they might perhaps be justly called lobar ducts. Owing, however, to the mechanics of development which crowd the gland into the small angle between the mandible and adjacent muscles the organ becomes distorted and its different portions have unequal opportunities for growth. Apparently these primary ducts have the same general caliber, although it is not uncommon to observe considerable variations in their diameter indicating that they drain unequal volumes of glandular

substance. Their number is not constant but varies in different glands between three and six. In the cases observed by the author there have been, as a rule, three ducts of the first order.

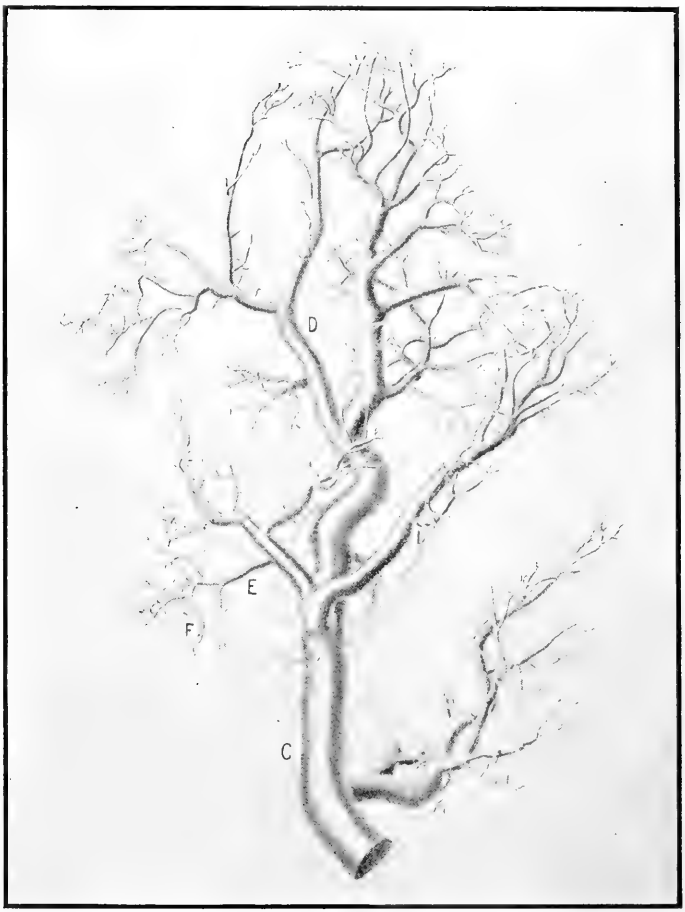

FIG. 2.—*Celloidin corrosion of an interlobular duct.* Magnified 12 diameters. The interlobular duct is shown taking a rather tortuous course and giving off sublobular ducts of the first and second order. From these the lobular ducts are derived and can be easily seen together with the larger portion of the intralobular system. The lobular ducts in proportion to their diameters appear rather long. *C*, interlobular duct; *D*, sublobular duct; *E*, lobular duct; *F*, intralobular duct.

From these main divisions arise the ducts of the second order which are usually termed the interlobular ducts. They are of large caliber, ramify extensively, and run for a considerable distance before giving

off the individual branches. There is often considerable tortuosity observed in their course (Fig. 2). They run between the lobules embedded in the thick fasciculated connective tissue of the interlobular spaces and are accompanied by the vasa comites. From these occasional lobular ducts are derived. As a rule, however, they break up into the sublobular ducts which leave the interlobular ducts at sharp angles and ramify among the lobules. They are called sublobular because it is from them or their chief divisions that the great majority of lobular ducts are derived. The latter are proportionately longer than ducts in other parts of the system and pass without dividing through the hilus of the lobule to ramify in the substance of the lobule itself. The position of the lobular ducts in corrosions can be identified by comparing them with the ducts of the same nature in digested preparations and sections of injected glands, the size of the ducts as well as their course corresponding perfectly in preparations made by both methods. When viewed under the stereoscopic microscope the lobular ducts ramify through three or four divisions which often follow in such close succession that the general rule of dichotomy seems to be violated. the case, for two complete trunks can always Careful study, however, shows that this is not be found after a division has taken place although they may occur very close together. The division is rapid so that the terminal ducts are thoroughly distributed throughout the lobule. These intralobular ducts which are synonymous with the salivary tubes of Pflüger, pass towards the center of the lobules and then radiate towards the periphery without ever quite reaching it, owing to the layers of acini which are interposed between them and the limiting membrane.

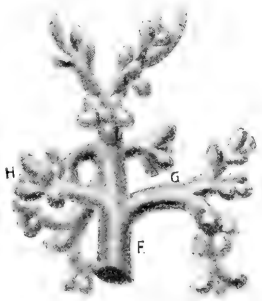

FIG. 3. *Celloidin corrosion of terminal ducts and alveolar ampullæ.* Magnified about 115 diameters. The main trunk in this preparation represents the end of one of the intralobular ducts which exhausts itself in giving off the intercalary branches. These may be divided once or twice and then terminate in the alveolar ampullæ which look like little ovoid or pear-shaped ends of the corrosions.

F—intralobular duct.
G—intercalary duct.
H—alveolar ampullæ.

When the terminal branches of the intralobular ducts are reached the law of dichotomy is often violated; branches occur more frequently and more abruptly, three or four sometimes arising at the same level. These divisions are the intracalary ducts which are usually about one-third the diameter of the terminal intralobular ducts in injected preparations. They run at obtuse angles from the ducts from which they spring. These intercalary ducts are of variable length and often

branch, although they not infrequently end in ampullæ without giving rise to a single vessel of a similar nature, especially in the mucous parts of the gland.

At the ends of the intercalary ducts are the ampullæ of the alveoli in which they terminate. These are surrounded by the secreting epithelium of the alveolus and represent moulds of the spaces into which the secretion is poured from the cells before it passes into the intercalary ducts. They have constricted necks marking the termination of the ducts and the end of this portion of the secreting system. Beyond the constriction their greatest diameter may be twice as large as the duct from which they spring. In corrosions they (Fig. 3) appear like ovoid knob-like endings to the intercalary ducts occurring in groups or clusters. As a rule they are slightly longer than they are wide, occasional ampullæ, however, are much longer than others, showing that they must have been derived from longer alveoli. In Fig. 3 the two apical ampullæ represent alveoli of this nature. Apparently the number of these structures arising from an intercalary duct varies between three and six, four perhaps representing the average number. This means, of course, that four alveoli, on an average, empty into each of the termini of the intercalary ducts. Whether they represent primary reservoirs for the storage of the products of glandular metabolism before they are emptied into the ducts it is as yet impossible to say.

In the course of development of the submaxillary gland the growing ducts, as we have already seen, are accompanied by blood-vessels which maintain throughout life this close and intimate association. Since blood-vessels follow in general certain laws of ramification, each trunk of the same size in an organ tending to give off an equal number of branches, it is not unreasonable to assume that the ducts may perhaps obey some similar law. Blood-vessels, of course, are not in stable equilibrium but are continually subjected to progressive and regressive changes which depend upon certain well-known laws.[1] The caliber of the vessel, for example, depends on the velocity of the current within it and this, in turn, depends partly on the nature and number of its branches. So far as we know the cross-section of the ducts is not the resultant of the action of any mechanical factors like those influencing the progressive and regressive changes in vessels, although it is by no means certain that some such mechanical control is not exerted. But even while it is true that there is a general tendency for ducts of the

[1] Thoma's Untersuchungen über die Histogenese und Histomechanik des Gefässsystems. Stuttgart, 1893.

same size to give rise to the same number of branches, this is by no means so definite and well marked as it is in the case of blood-vessels. In the submaxillary gland the following general quantitative relations are found in the successive ramifications of the ducts.

The Ductus Submaxillaris divides into
3 Primary Ducts which divide into
18 Interlobular Ducts which divide into
96 Sublobular Ducts which divide into
1500 Lobular Ducts.

Obviously a table of this nature must be interpreted liberally inasmuch as it indicates only the average scheme of division in a system which varies within wide limits. As an absolute standard it is worthless, its chief service being to indicate the general plan of ramification of the ducts, estimated from corrosion preparations of several glands. Since the lobules are always drained by a single duct we find from the above table that there must be approximately 1500 lobules in the entire submaxillary gland.

In the corrosions one is often struck by an apparent similarity between the ducts of the lobule and the ducts of the gland as a whole, the former appearing much like a miniature reproduction of the latter. When attention is called to this analogy it becomes immediately patent and may, indeed, be extended to many other features of the gland as we shall have occasion to show later. The duct enters the gland at the hilus; the lobular duct passes into the lobule through a similar portal. There is but one submaxillary duct to each gland; there is likewise but one lobular duct to each lobule. In the gland the ducts ramify through the central portion without ever reaching the capsule, keeping, indeed, as far away from it as the anatomical conditions which require the drainage of the whole gland will permit. In the lobule the intralobular ducts take the same course with reference to the Membrana limitans and and the drainage of the lobular alveoli. This analogy may be of more interest than importance. Its explanation affords no difficulty since the lobular and intralobular ducts are formed by the same laws of growth and mechanics of development which give rise to the larger ducts of the gland as a whole.

The Ducts in Sections and Digested Preparations.

In preparations made by the method of piece digestion which have been cleared by glycerine, xyol or creosote, the form of the lobule, the frame-work, and particularly the distribution of the interlobular and

intralobular ducts, can be easily seen. The relations of the ducts to these structures are likewise sharply defined so that one obtains by the use of the stereoscopic microscope the relations of the vascular and secretory units to the frame-work and the structures of the gland. In these specimens the interlobular septa and their relation to the capsule can be easily determined. The larger ducts and vessels in the interlobular septa are readily followed, owing to the difference in diffrac-

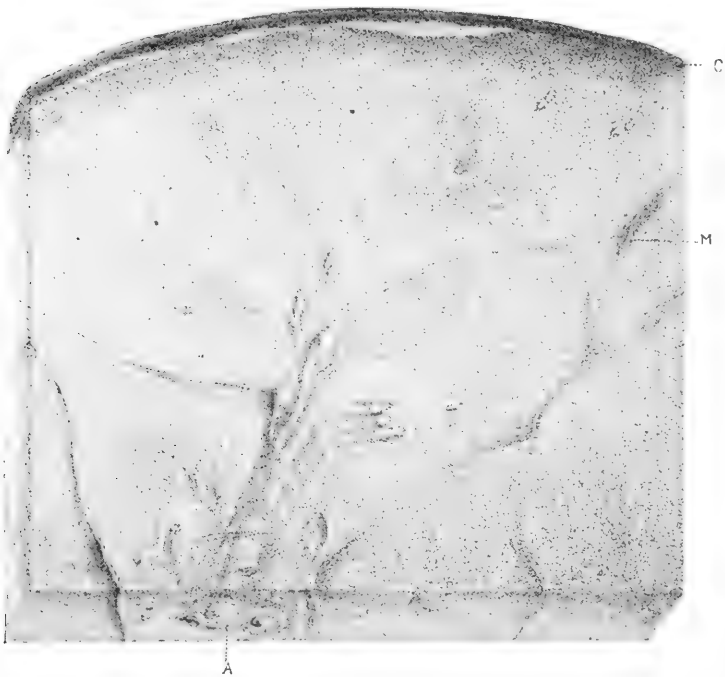

FIG. 4—*Piece digestion of dog's submaxillary.* Magnified 10 diameters. This specimen shows the sublobular interspaces and the passage of lobular ducts and connective tissue from the interspace into the lobule through its hilus. The relation of the membrana limitans to other lobules is shown as clearly in this specimen as in figure 8. The course of the intralobular ducts is plain as they pass through the fine supporting meshwork formed by the basement membranes of the alveoli. *A*, Sublobular interspace with artery, duct, and veins; *M*, Membrana limitans; *C*, Capsule.

tion between them and the frame-work of other portions of the organ. The ducts, vessels, and septa appear in these specimens, when viewed by transmitted light, considerably darker than the fine lobular frame-work in which they run and they can be easily distinguished from each other by their size. The ducts are considerably larger than either of the vasa comites which run in the same interspace. Embracing the

group of vessels are fine fasciculated bands of connective tissue which form the interlobular spaces. These can be made out in digested specimens both by the position of the vessels and the darker areas which they produce as they pass between the lobules. Fig. 4 is a representation of the submaxillary of a dog prepared by this method. The ducts and vessels lie in a sublobular interspace embraced by the connective tissue which is seen in cross-section at the edge of the block of tissue. The larger duct in this space runs for only a short distance before entering the lobules and is, therefore, of the order of sublobular ducts. The lobular ducts which are given off from this branch pass through the hilus of the lobules carrying with them considerable connective tissue derived from the interspace from which they come (Figs. 4 and 5). After penetrating the lobule they run to the center of that structure and then begin to radiate towards its periphery. The intralobular ducts can be distinguished from the blood-vessels by their caliber and the delicacy of the walls. Isolated terminal branches are seen towards the periphery of the lobule, but in no instance does a duct ever seem to reach the limiting membrane, a layer of one or two alveoli always intervening. The frame-work within the lobule and its relation to the ducts is exquisitely shown in these preparations; the strands of connective tissue entering at the hilus, the fine delicate limiting membrane embracing it, and the basement membranes of the alveoli are all patent, the latter appearing like a delicate web throughout the whole lobule. This is firmly attached to the limiting membrane on the one hand where it forms a sort of mosaic, and to the walls of the ducts and vessels on the other, so that the support of these structures is given by the alveolar frame-work, and they are, as it were, swung in the meshwork of delicate reticulated basement membranes.

In ordinary sections, the Ductus submaxillaris is lined by a double layer of epithelial cells, the inner of which is irregularly columnar and has oval nuclei which stain deeply and show distinct gatherings of chromatin substance upon the linin filaments. These cells interdigitate with those of the outer layer which are more conical and polyhedral in shape, and are, as a rule, considerably smaller. The nuclei of the latter are smaller, somewhat more deeply stained and often more vesicular in shape. The two layers of epithelial cells rest upon the basement membrane of the duct which is immediately embraced by the fasciculated intertwining strands of white fibrous tissue and reticulum. Immediately external to the epithelial layer of basement membrane is a dense meshwork of elastic fibres which interlace with the fibres of reticulum and white fibrous tissue. The few smooth muscle fibres described by von

Kölliker can be seen in specimens stained by Van Gieson's method, and the lumina of the rich plexus of arterioles, venules and capillaries that surround the duct are often seen in cross-section. Ducts of the first order present no characteristic differences from those observed in the Ductus submaxillaris, except that the connective tissue which em-

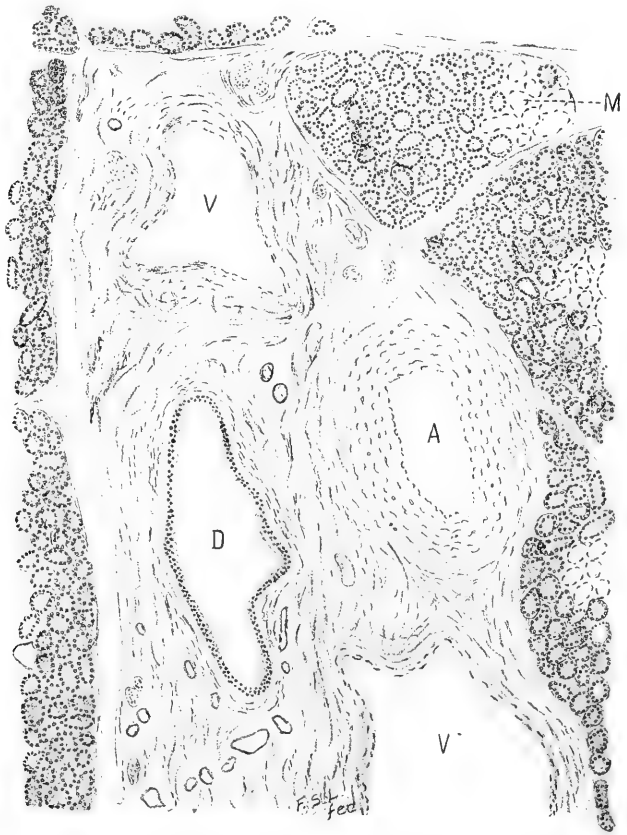

Fig. 5.—*Sections of a human submaxillary gland stained by Henson's modification of Van Gieson's stain.* Magnified about 85 diameters. This section shows one of the interlobular spaces with the duct and its vasa comites. Adjacent lobules show the mucous and serous portions of the gland. *D*, Interlobular duct; *V*, Interlobular vein; *M*, Mucous alveoli; *A*, Interlobular artery.

braces them is far richer owing to the fact that it now carries not only the excretory channels and the blood-vessels of the organ, but, in addition, forms the main interlobular support of the gland as a whole. This connective tissue is continuous with that which enters the gland at the hilus and forms the main support of the glandular lobules. The

interlobular spaces in the submaxillary gland may be compared to those in the liver, except that in the case of the former we have usually two veins accompanying the duct instead of the single branch of the portal vein which we are accustomed to see in the liver. The interlobular

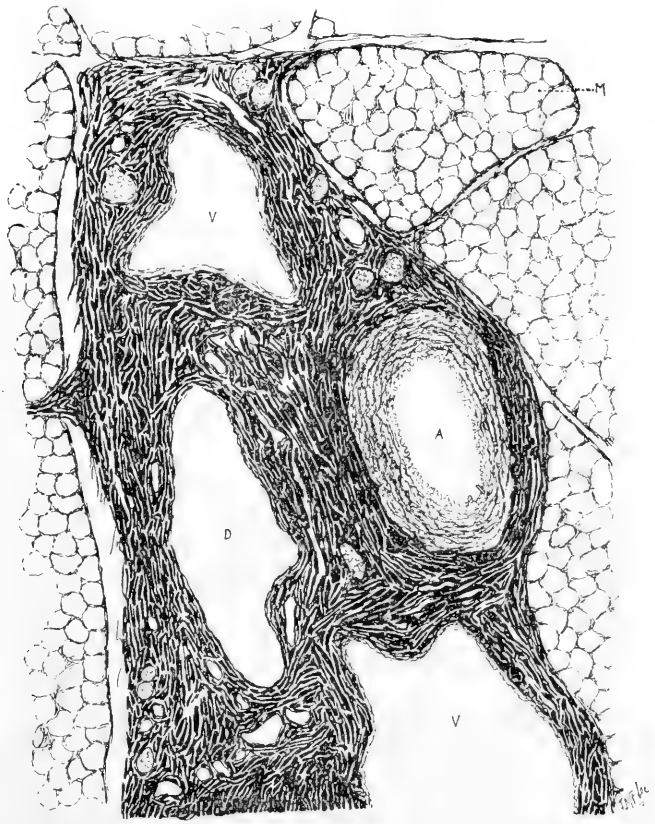

FIG. 6.—*Slide digestion after Spalteholz.* Magnified about 85 diameters. The section is the one just following that shown in figure 5. All of the cells have been removed from the specimen which shows fasciculi in the interlobular spaces and the basement membranes about the alveoli. It is at once apparent that without the control specimen it would be impossible to distinguish the sections of the intralobular ducts from the alveoli. The basement membranes of both structures have practically the same arrangement. *D*, Interlobular duct; *A*, Interlobular artery; *V*, Interlobular vein; *M*, Mucous alveoli.

ducts (Fig. 5) like the other main channels are lined by two layers of epithelial cells which possess all the chief characteristics of those we have just described in ducts of a lower order. They also rest on the basement membranes. In preparations that have been digested on a

slide (Fig. 6) this basement membrane can be distinguished at the inner edge of the lumen of the duct where it appears as a delicate irregular line. The slight clear area just outside of the basement membrane is caused by the spaces left by the elastic tissue, the fibres of which have been entirely dissolved from the specimens by the action of the enzyme. The connective tissue embracing the duct is now distinctly fasciculated and arranged so that its bundles seem, at the same time, to give the greatest strength and elasticity to that part of the duct. Numerous connective tissue corpuscles, endothelial cells lining the lymph spaces and capillaries, can be seen in the frame-work just about the interlobular ducts, the vasa comites cut in cross-section are also evident. Specimens stained with Weigert's elastic tissue method (Fig. 7) show external to the basement membrane, a dense, deeply-staining, elastic membrane, composed of interlacing elastic fibres which entirely embraces the duct and appears, in these specimens, like an irregular black line just external to the epithelium. Numerous elastic fibres having a concentric lamellar arrangement are found outside of the main elastic membrane. Some fibres connect the different concentric elastic lamellæ, while others, variously arranged, appear to be extensively distributed throughout the entire interspace. Ducts of the next higher order, namely the sublobular ducts (Figs. 4 and 8), are likewise embraced by the connective tissue of the sublobular spaces, but this is now greatly diminished in amount. The sublobular ducts like those of the lower orders are lined by a double layer of epithelial cells. The cells of the inner columnar layer are much lower than those of the corresponding layer of interlobular ducts or ducts of the first order. The nuclei are more nearly spherical, the cytoplasm is somewhat diminished in quantity and appears slightly more granular. These cells are likewise slightly smaller and more compact than those of the corresponding layer of the larger ducts, the basement membranes are clearly marked and the connective tissue has the same characteristic fasciculated appearance noted in the larger interspaces, except that the fasciculi are much smaller and more compactly arranged. Connective tissue cells, endothelial cells and blood-vessels are also found in these spaces bearing ostensibly the same relation to the ducts and connective tissue which we have observed about the larger branches.

The elastica of the sublobular ducts is very well marked, forming a thick mesh-work of anastomosing and interlacing fibrils lying just beneath the membrana propria. As in the larger ducts there is the same concentric arrangement of the elastic lamellæ, the latter alternating with layers made of white fibrous tissue and reticulum with numerous

coarse elastic fibres running in between the elastic tissue bundles. In the meshes of the elastica are found numerous bundles of ordinary fibrous tissue so that in digested specimens the position of the elastica,

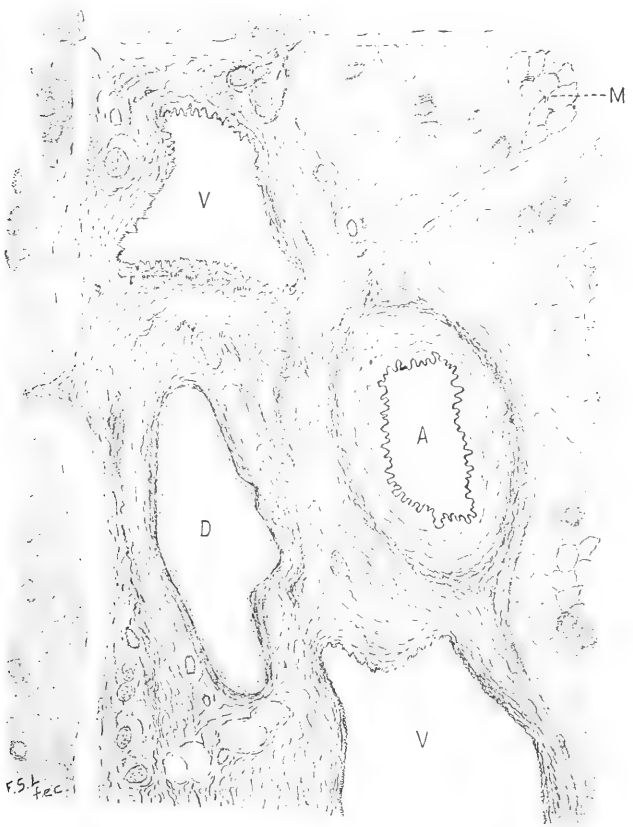

FIG. 7.—*Section preceding the one shown in figure* 5.—*Stained by a modified Weigert's elastic stain.* Magnified about 85 diameters. The elastic membrane, the concentric lamellæ about the interlobular duct are clearly shown together with the elastic fibres scattered throughout the interlobular space. The characteristic arrangement of the fibres about the blood-vessels can also be noted. In comparing this figure with the preceding one the mucous alveoli are seen to be surrounded by a delicate elastic membrane while only occasional fibrils are found about either the serous alveoli or the intralobular ducts. *D*, Intralobular duct; *V*, Intralobular vein; *A*, Intralobular artery; *M*, Mucous alveoli.

even though it has been dissolved by the enzymes, is indicated by the fact that the frame-work is more open at the points previously occupied by the elastic fibres.

As the duct enters the hilus of the lobule (Fig. 8) a considerable portion of the sublobular connective tissue is carried in with it, a relation between the lobular duct and lobule similar to that observed between the Ductus submaxillaris and the gland as a whole. The connective tissue which enters the lobule at this point, is continuous with that of the sublobular space from which it springs and possesses ostensibly the same characteristics and relations. It may be observed that the spaces containing the sublobular ducts are the points of origin of several lobules which may be seen in sections and piece digestions as arising from these centers (Figs. 4 and 8). That is to say, the hilus of the lobule is attached at these points and this intimate association with the sublobular interspaces is the only firm point of union between the lobules and the glandular frame-work, inasmuch as the fibrils connecting adjacent limiting membranes are, as a rule, too scant and delicate to have an extensive supporting function (Figs. 5 and 8). This is a point of considerable importance when the origin of the lobule in the development of the gland is considered. As soon as the duct is within the lobule the same relations observed in corrosions and digestions are seen in ordinary sections, namely that the ducts which are cut in various directions, transversely, longitudinally and obliquely, are observed to lie in the central portions of the lobule away from the membrana limitans. Indeed, it is only very rarely that sections of the ducts are seen nearer the perilobular membranes (Fig. 8) than the

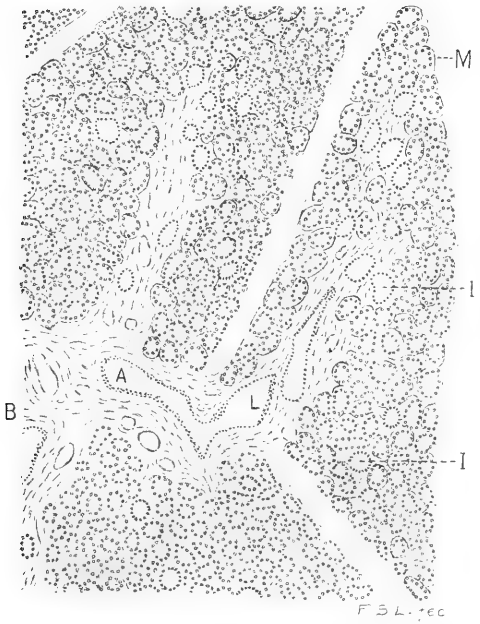

FIG. 8.—*Lobule of the human submaxillary gland, showing the hilus and the lobular ducts.* Magnified about 85 diameters. Same stain as Fig. 5. The attachment of the lobule to the sublobular interspace is clearly shown and the arrangement of the connective tissue as it enters the lobule with the lobular duct, is evident. The membrana limitans has very few connections with those of the adjacent lobules, the main attachment of the lobule being the portion at the hilus. The distribution of the intralobular ducts in the central portion of the lobules away from the membrana limitans is well represented. *A*, Sublobular duct; *B*, Sublobular interspace; *M*, Membrana limitans; *L*, Lobular duct at hilus of lobule; *I*, Intralobular duct.

width of two alveoli. Sometimes they may approach as near as one alveolus but an instance of a duct lying adjacent to the limiting membrane is almost impossible to find, except at the hilus, where the lobular duct enters the lobule. Once in the lobule the duct loses its double layer of epithelium and is lined from this point with a single layer of short columnar or cubical cells which are characteristic of the ducts in this region. These intralobular ducts are known throughout the literature as the "salivary tubes of Pflüger" who believed that they were concerned in the metabolic activities of the gland and took an active part in the phenomena of secretion. These cells have oval or vesicular nuclei which are situated about the center of the cells. As a rule they take the stain somewhat more deeply than the nuclei of the epithelium in the extralobular ducts. The portion of the cell towards the lumen of the duct is composed of granular cytoplasm which stains deeply with the ordinary acid contrast dyes, such as congo red, eosin, or the picric acid element of the Van Gieson stain. The pole of the cell external to the nucleus shows a characteristic appearance of longitudinal striations which run from the central portion just below the nucleus to the end of the cell near the basement membrane. In cells that have been isolated from the ducts the portion of the cytoplasm occupied by these striations splits into little staves which often spread out much like the sticks of a fan. Protoplasmic bridges have been described running between the individual filaments composing these striations.

As we have seen from the corrosions, the intercalary ducts form the termination of the intralobular ducts and connect them with the alveoli. They are seen readily in sections where they appear when undistended only about one-third of the diameter of the intralobular ducts. The epithelium of these structures changes suddenly from the striated cubical cells and is composed of rather long, flattened epithelium cells with their major axis running parallel to the axis of the duct. The nuclei are elongated, less deeply staining than the nuclei of the cells in ducts of the next lower order. The cytoplasm is neither so rich in quantity nor does it have the same affinity for the acid dyes that we have observed in the cells of the intralobular ducts. The boundaries of the cells, moreover, are somewhat obscured. When cut in cross-section the lining cells of these ducts appear as flattened cuboidal epithelial elements. There is little elastic tissue about either the lobular or intralobular or intercalary ducts, only an occasional fibril can be made out surrounding them. The regular elastic membranes described in the extralobular system cease shortly after the ducts become intralobular. Sometimes, however, they may be observed following the

lobular ducts for some little distance into the lobule, but these cases are exceptional. Both the interlobular and intercalary ducts are provided with basement membranes which differ so little from the membranæ propriæ of the acini that it is usually necessary to orient them in digested specimens in order to distinguish ducts from alveoli (Fig. 6). These basement membranes consist of a delicate network of interlacing fibrils of reticulum which appear in sections cut tangentially to the membrane as a cross-hatch or mesh-work of interlacing fibrils which are only visible with the immersion lens. When viewed with the lower powers the membrana propria usually appears practically homogeneous.

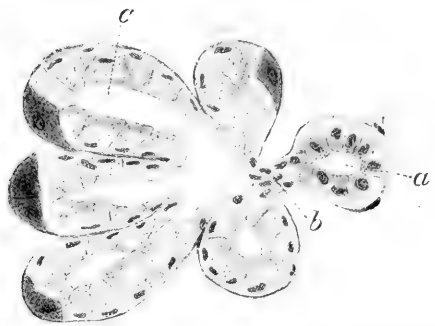

Fig. 9. Terminal intralobular duct, intercalary duct, and group of mucous alveoli showing the alveolar ampullæ. Human submaxillary gland. Stained by Henson's method. Magnified 300 diameters.

The direction of the section is such that the intralobular duct is cut in cross section and the intercalary duct tangentially so that one does not see its lumen. Inasmuch as the alveoli are not collapsed, the alveolar ampullae are distinctly visible.

a—intralobular duct.
b—intercalary duct.
c—alveolar ampulla.

At the point of termination of the intercalary ducts a slight constriction is noted, in corrosions, just as they widen into the ampullæ. In sections the flattened epithelium at this point changes abruptly into the regular epithelium of the alveolus. In sections cut in the right plane it is possible not only to see the intercalary duct terminating in the alveoli but to make out the little ovoid spaces forming the ampullæ as well (Fig. 9). As a rule, however, the inner borders of the cells of the alveolar epithelium are in close approximation so that in the collapsed state of the alveolus only a small chink is left between them. About the serous alveoli there are only a few occasional elastic fibres; about the mucous alveoli, however, these fibres are numerous, as has been shown by Livini.[8] Their nature and relations, however, Livini did not describe. It appears that these fibres, which under the lower power of the lens look homogeneous, are in reality very delicate elastic membranes made up of an intertwining and interlacing mesh-work of fibres which have a reticulated appearance similar to that of the regular membrana propria. This elastic membrane appears to lie outside of the regular membrana propria of the mucous alveoli. As Livini pointed

[8] Livini: Monitore Zoologico Italiano, vol. x, 1899.

out, the thick, ropy, tenacious secretion of the mucous alveoli is partly expelled from the alveolus into the ducts by the assistance of the mechanical action of this elastic membrane. He did not suggest, however, the interesting corollary that, in the secretion of this substance, the elastic tension of this same mebrane must be overcome, which means that secretion in the mucous alveoli must at least be accomplished under a sufficient pressure to overcome this elastic tension. It is, of course, well known that secretion takes place under a pressure higher than that of the blood, and this, together with the recent work which seems to indicate that the osmotic pressure within the cell is twenty times greater than the blood pressure, would explain how the stretching of this elastic membrane could be easily accomplished during the activity of the glandular cells.

There are several characteristic staining reactions of the duct epithelium which can be observed with more or less distinctness from the Ductus submaxillaris to the alveoli. The duct cells take the ordinary contrast stains deeply. They exhibit especially a peculiar affinity for congo red. Accordingly as in the case of demilunes of Gianuzzi, congo red may almost be considered as the special selective stain for the duct epithelium. When elastic tissue preparations are made and contrasted with picric acid the epithelial cells of both intra- and extralobular ducts take a rather yellowish-green tint, while the rest of the epithelium is only a pale yellow (Fig. 7). In Van Gieson preparations or modifications of this method the duct epithelium stains a pale yellow while the serous alveoli are a deep purple and the mucous alveoli stain a dark blue.

Discussion of the Literature.

Comparatively little work has been done upon the ducts of the salivary glands alone, most of the research appearing as collateral study in course of work upon other portions of the organs. Von Kölliker[9] states that the ducts of the salivary glands are made up of a single layer of cylindrical epithelium which is surrounded by connective tissue and some elastic fibres. Those about the D. submaxillaris according to Kölliker are arranged in the form of a double membrane. As we have seen, however, there is just one well-marked elastic membrane located external to the membrana propria and several concentric, less regularly arranged lamellæ situated external to the regular elastic tunic. It was one of these, no doubt, which von Kölliker believed was the second elastic sheath. He states, moreover, that this double arrangement of

[9] Kölliker: Gewebelehre. Bd. ii, Leipzig, 1852.

the elastic fibres was limited solely to the main duct, whereas by means of improved elastic tissue stains we can show that these concentrically arranged elastic fibrils embrace ducts of all orders as high as those which drain the lobules.

According to the Tobiens[10] the ducts of the glands in general consist of connective tissue. Those of the salivary glands in addition possess muscle fibres, which are arranged in an outer longitudinal and an inner circular layer. All the ducts of man, horse, dog, and cat have, according to this investigator, elastic fibres which vary inversely with the amount of muscle present. The arrangement of the fibres is inconstant, but there is usually an inner circular layer, while in man spirally arranged fibrils situated outside of this layer can occasionally be found. The results of Tobiens' work, however, has never been confirmed.

Krause[11] describes the ducts as consisting of fine-meshed connective tissue with numerous longitudinal or transversely-running elastic fibres. With the exception of Wharton's duct, muscle fibres do not occur in the walls of the submaxillary ducts. Previous to the work of Henle[12] the epithelium of the Ductus submaxillaris has always been described with a single layer of epithelium. He states definitely, however, that the epithelial lining of Wharton's duct is made up of a double row of cells. This observation has now been shown to be true of all the ducts of the extralobular system as well.

Von Ebner[13] supports the work of Pflüger on the structure and nature of the salivary tubes and describes, for the first time, the intercalary ducts. These, he states, are clothed by cubical epithelium and form that portion of the excretory system between the alveoli and the salivary tubes of Pflüger.

This fact was emphasized by Klein[14] and Heidenhain.[15] Klein described the epithelium of the ducts of the human submaxillary as consisting of an inner layer of cylindrical cells with long nuclei and a deeper layer of small cells with oval nuclei. In his later paper Klein goes extensively into the origin and relation of the ducts. Among other things he states that the amount of connective tissue supporting

[10] Tobiens: De glandularum ductibus efferentibus ratione imprimis habita te; ae muscularis. Inaug. Diss. Dorpat, 1853. Cited by Oppel.

[11] Krause: Zeit. f. rat. Med. Bd. 21, 1864.

[12] Henle: Eingeweidelehre, 1871.

[13] Von Ebner: Arch. f. mik. Anat., Bd. VIII, 1872.

[14] Klein: Quar. Jour. of Mic. Science, N. S., vol. XIX, 1879.
Klein: Quar. Jour. of Mic. Science,, vol. XXII, 1882.

[15] Heidenhain: Hermann's Handbuch d. Physiologie, Bd. V, 1880.

the interlobular ducts and vessels is subject to considerable variation in different glands, but in all instances this connective tissue is proportionate to that penetrating into the interior of the lobules with the chief ducts and vessels. In sections of the submaxillary of man the connective tissue around the larger ducts and vessels appears to be of the same nature as in the organs where the fibrous tissue is arranged in continuous and compact masses, that is to say groups, bundles, or trabeculæ of fibrous tissue running in various directions are seen cut at various angles. Between these fasciculi are the interfascicular spaces more or less dilated according to the method of hardening. Among the connective tissue fibrils Klein found branched cells, plasma cells of Waldeyer, and some *Mastzellen* of Ehrlich. The important point is that the groups or bundles are arranged into definite plates which vary greatly in breadth and thickness. These Klein calls the fasicle plates, each of which is composed of a number of fasciculi or bundles of connective tissue fibrils which are continued into the lobule in company with the lobular ducts. The interlobular ducts in most glands, according to Klein, are lined by a double layer of cells, the inner of which are cylindrical and the outer, next to the membrana propria, conical. The cytoplasm of these cells shows occasionally a tendency to fibrillation similar to the striations observed by Henle and Pflüger in the intralobular ducts. This observation, however, has not been repeated by other investigators nor is supported by the preparations used in this study. The intralobular ducts are lined by a single layer of columnar cells, showing the characteristic fibrillations which are joined by short lateral branchlets, and, therefore, converted into reticulum. Distinct from these are the spindle-shaped or staff-shaped cells which are in communication with the membrana propria and extend from this structure up between the epithelial cells, and in some cases, form a sort of inner membrane within the lumen. Klein states that these cells are particularly well marked in the parotid gland of guinea-pigs. No other investigator, however, has described their existence nor have they been found in the ducts of the human submaxillary. As the intralobular ducts pass over into the intercalary portion there is a distinct shorter portion which Klein calls the neck. This is characterized by the lumen and the whole breadth of the salivary tube becoming here suddenly smaller. In the submaxillary of the pig Klein says there is no intercalary portion, in the submaxillary of man he finds in the serous portions a short neck passing over into a long, fine, intermediary duct, while in the mucous parts the neck terminates directly in the alveoli.

Kultschultzky[16] describes in the epithelium of the intralobular ducts of the submaxillary of the hedge-hog three distinct cytoplasmic zones, an inner mucous zone, a protoplasmic zone, and a rodded zone next to the membrana propria. In the human gland three zones can be distinctly seen, but whether the deeply-staining portion adjacent to the lumen is due to a mucous zone has not yet been definitely settled.

Toldt[17] divided the excretory system into three portions, branches extending from the hilus of the gland to the points where they enter the lobule, branches given off within the lobule, and finally the so-called intercalary ducts connecting the alveoli with Pflüger's salivary tubes. These ducts, according to Toldt, do not have the same arrangement in all glands and may even vary in the mucous and serous alveoli of the same gland. Toldt first called the attention to the fact that the nature of the division of the ducts is dichotomous and that this plan occurs both within and without the lobule.

According to Krause,[18] the height of the epithelium depends on the diameter of the duct, the one varying directly with the other. He shows also in one of the figures, viz., Fig. 7, an ampulla within the alveolus but does not seem to recognize its importance as a definite part in the secretory system. In the schema given in Fig. 10, Krause also represents long serous alveoli given off from the intercalary ducts; the regularity of the ovoid ampullæ found in corrosion preparations shows that the alveoli of both mucous and serous portions possesses also this same general shape, with perhaps a more marked constriction at the end where they rise from the intercalary ducts. This fact is also emphasized by the work of Maziarski,[19] who has used in two splendid researches on the classification and structure of different glands, Born's wax-plate method for the reconstruction of their terminal ducts and alveoli. Among other glands, both mucous and serous portions of the submaxillary were studied by this investigator. The results show that the salivary tubes or intralobular ducts in the serous parts of the gland break up after a short course into the intercalary ducts. These subdivide again until they terminate finally in the alveoli. The alveoli are slightly oval or pear-shaped and look like a bunch of grapes hanging on a stem. In the mucous portions of the gland the intralobular ducts seem larger than the serous parts. The intercalary portion which is also

[16] Kultschultzky: Zeit. f. Wiss. Zool., XLI, 1885.

[17] Toldt: Gewebelehre, Stuttgart, 1888.

[18] Krause: Arch. f. Mik. Anat., Bd. XLV, 1895. Bd. XLIX, 1897.

[19] Maziarski: Bull. Internat. de l'Acad. d. Scien. de Cracovie, 1900. Anat. Hefte, Bd. XVIII, Heft I, 1901.

smaller forms, according to Maziarski, the duct for the whole group of alveoli.

If one were to take a piece of the corrosion, as for example, Fig. 3, and clothe different portions with the epithelium which they normally possess, a picture would be obtained in this way almost identical with that given by Maziarski, except that owing to the regular distension of the system with the injection mass the form in this case would be somewhat more regular than that observed in Maziarski's reconstruction. The latter did not recognize the ampullæ within the alveoli as they were collapsed by the fixation of the tissue, and therefore, are not rendered patent except, perhaps, in exceptional cases.

In conclusion, I wish to express my great indebtedness to Dr. Revell, of the Department of Anatomy of the University of Chicago, for the drawings of the corrosions.

ON THE SKELETON OF NYCTODACTYLUS, WITH RESTORATION.

BY

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WITH 1 TEXT FIGURE.

The genus *Nyctodactylus* was proposed by Marsh, in 1876, for a pterodactyl from the Niobrara cretaceous of Kansas. Though very inadequately described, the single distinctive character given by him—the non-articular distal extremity of the scapula—permitted its recognition with certainty, and, in 1892,[1] I gave additional characters placing the genus on a secure foundation. A specimen of this genus, of unusual perfection, recently collected by my assistant, Mr. H. T. Martin, in his usual skilful manner, presents so many interesting new features that I give herewith a brief description of its more important characters in advance of a monographic study of the group, which I hope to find time to undertake soon. The specimen is very nearly complete, lacking only the two distal phalanges of the wing finger in part, and many of the small bones of the digits and of the tail. The skeleton lies upon its back, with but little distortion or disarrangement of the bones; the right wing is folded across the abdomen, the neck vertebræ are partly dislocated, and the legs have been drawn a short distance away from the pelvis. The head lies obliquely to the long axis of the skeleton, with its palatal surface uppermost; and the bones of the pelvis have been separated at the sutures, lying flattened out with the sacrum in the middle. The outlines of the different parts have been, for the most part, made from tracings; that of the skull has been taken in part from a specimen of *Ornithostoma* in the museum, since the skull of the specimen is too delicate to remove from the matrix; its anterior portion, however, has been examined on both sides, as also the mandible, and I do not think that the shape as given can depart much from the reality. I may further add that the feet and small fingers have been completed from specimens of *Ornithostoma*. Because the bones of these pterodactyls are so ex-

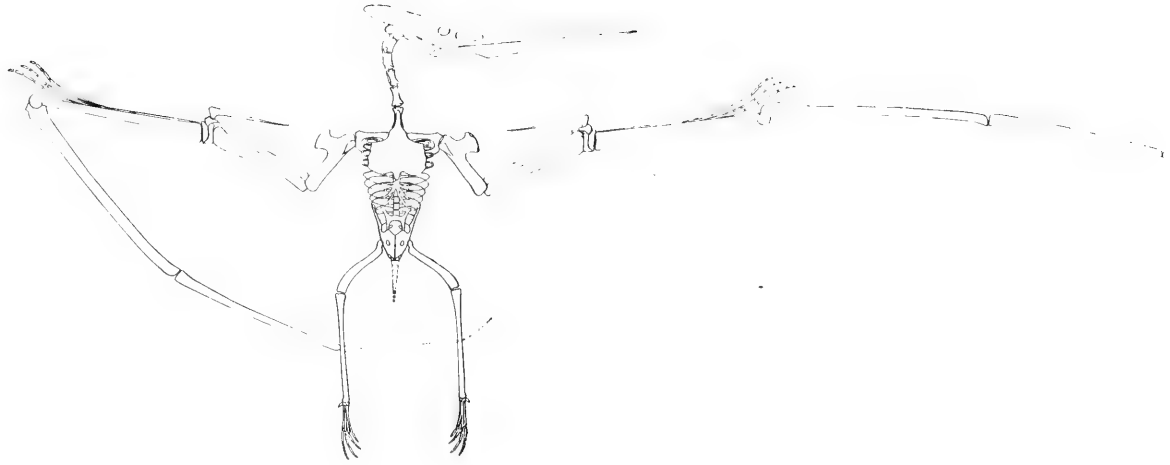
[1] Kansas Univ. Quart., Vol. I, p. 12.

ceedingly thin they have been invariably flattened out in fossilization; the first phalange of the wing finger, the longest bone in the skeleton, has a thickness at its middle, as crushed, of less than one and a half millimeters. On account of this flattening, it is difficult to estimate accurately the real width that the bones had in the living skeleton; possibly they are represented in the drawing, for the most part, too broadly.

A brief resumè of the more important osteological characters of this pterodactyl may now be given as follows:

Head long and slender, toothless; antorbital opening confluent with the nares; atlas and axis partly or entirely cöossified; seven true cervical vertebræ present, without free ribs, and with non-articular exapophyses.[2] Eighth vertebræ apparently ribless, much shorter than the seventh, with the posterior zygapophyses much prolonged; ninth, tenth and eleventh vertebræ, that is, the second, third and fourth dorsals, coössified, and each with stout coössified ribs, or much elongated diapophyses, articulating with the sternum; fifth to ninth dorsals, inclusive, short, stout, procoelous, with elongated diapophyses; tenth, and perhaps also the ninth (which is partly concealed beneath the radius), coössified with the sacrum; sacrum composed of six firmly fused vertebræ, all united with the ilium, and tapering much distally; caudal vertebræ amphiplatyan, probably about twelve in number (the first one and the three distal ones, only, so far discovered in the specimen). Ilium projecting far in front of the sacrum, narrow; ischium (or conjoined ischium and pubis) with a long, somewhat arcuated median symphysis, and with a large obturator foramen; acetabulum imperforate, situated far dorsad; prepubis (pubis?) band-like, with an anterior projection, U-shaped in life. Sternum very broad and thin, evidently deeply concave above, without keel, but with a stout presternal process; with four costal articulations on each side, and a median, flattened, xiphisternal process. First three or four dorsal ribs stout, coössified with the vertebræ, and articulating with the sternum; posterior ribs very slender, almost thread-like, probably articulating in front with the extremities of the abdominal ribs, single headed; abdominal ribs at least four in number on each side; arranged very much as are the costal cartilages of the sixth to the tenth ribs in man, but joined in front and attached to the xiphisternal process.

[2] Plieninger, (Paleontographica, XLVIII, 82, 1901) objects to this term, and identifies the processes with the parapophyses. Assuming that they are morphologically identical with the real parapophyses, which is by no means proven, and is to me very doubtful, their very different position and function necessitate a distinctive name, for which I proposed that of exapophyses (Kans. Univ. Quarterly, 1896).



RESTORATION OF SKELETON OF NYCTODACTYLUS × $\frac{1}{16}$.

S. W. Williston.

Coracoid and scapula coössified (imperfectly so in another specimen of the same species), the former articulating by the usual saddle-shaped joint with the sternum, the latter terminating in a free, spatulate extremity, without union with the notarium. Humerus with its deltoid process very long, helmet-shaped and with a constricted neck; remaining bones of the extremities very much as they have been described in *Ornithostoma*.

In the restoration given herewith, in which the measurements have been made with great care by myself, one is struck with amazement at the extraordinary development of the head and wings as compared with the rest of the skeleton. While the wings gave a spread of very nearly eight feet, the body proper was less than four inches in diameter and not more than six in length, exclusive of the small tail; the pelvis is less than five-eighths of an inch in diameter at its outlet, and the entire body was smaller than one's closed hand! One wonders where sufficient surface was presented for the attachment of the strong muscles necessary for the control of the wings. When it is remembered, however, that even the largest bones of the skeleton had walls less than a millimeter in thickness, and that many of the smaller ones were almost like cylinders of writing paper, he will perceive that, notwithstanding the extraordinary development of the anterior extremities and head, the creature, when alive, must have weighed but little. I very much doubt whether the living animal attained a weight of five pounds. How and where such creatures could have reared their young is to me inexplicable. No evidences have been found in the many specimens of these animals that have been exhumed from the Kansas chalk that they were viviparous, and from the high degree of ossification of the bones in the adult, it is quite sure that the fœtus must have had a bony skeleton, and that evidences of such would have been forthcoming before now had the young really been born alive, unless, indeed, in the immature condition of marsupials. If eggs were laid, they could not have been more than a centimeter in diameter, and even if much elongated to accommodate the long bones of the wings, the newly hatched pterodactyl could hardly have been of sufficient size to have cared for itself.

A number of other interesting conclusions—or speculations—are suggested by the present specimen. The acetabulum is placed far back, nearly over the edge of the sacrum; so far back, indeed, that it would have been impossible for the knees to have met in the middle, when the thighs were flexed to a right angle. Furthermore, the femora have a peculiar mesial convexity, whereby the tibiæ were directed at a marked angle outward, with the thigh in the normal human position. The convexity

of the head of the femur, covering a little more than a third of a circle, is at right angles to the long axis of the shaft, making articulation impossible, except in a strongly abducted condition. Similar evidence is presented by the glenoid articulation of the humerus, demonstrating, I think, the improbability of an ordinary quadrupedal position in ambulation, as Seeley has restored some of the European forms. I am convinced that the thigh was rotated outward, through an angle of thirty or forty degrees, and was directed outwardly nearly in a coronal plane at an angle of thirty or forty degrees from the mesial line. I am not sure, indeed, but that the knees may have been turned at times more or less backward. The condylar surface of the femur is such that the knees could not have been flexed much, if any, more than a right angle. The tibiæ might easily have been brought parallel with each other, while the femora were outwardly rotated and abducted.

This normal outward rotation of the femora is also shown in an excellent specimen of the hind extremities of *Ornithostoma ingens* preserved in the matrix. The heads of the femora occupy their normal relation to each other in connection with the remains of the pelvis, but both were directed outwards with the trochanters turned inwards. I may add here that in removing one of these femora for further study of its shape, I was greatly surprised to find on the under surface—the ventral—very vivid markings of the integument. Photographs of these will be given later. I may say here that there is no direct evidence of either scales or feathers, but the numerous, regularly placed patches of darker material are such as might have been produced by the skin of a bird where there are many feathers. Since we have hitherto been entirely ignorant of the covering of the body of these animals, the discovery is one of great interest. I am convinced that the integument was not a simple smooth membrane over the body, though what it really was I am not prepared to say. I expect to find further evidence, that I hope will solve the question, when the remaining bones of the specimen have been removed from the matrix.

So far, then, as the evidence of the legs goes, the animal may have stood erect upon its feet with the thighs rotated outward and tibiæ far apart. Of the clawless character of the feet in these animals there can be no question. A specimen collected by me nearly ten years ago has the bones all intact and in position. The feet were not in the least prehensile.

The articulation of the humerus with the coraco-scapula was nearly perfectly saddle-shaped, with its axes nearly in the planes of the body. Unlike the legs, there was little or no rotation of the upper extremity,

either in the shoulder joint or elsewhere. This, it will be seen, must have detracted not a little from the ability to control the direction of flight by the wings, while giving greater strength to them as parachutes. The wing membranes could never have have assumed any form, except that of an approximate plane when distended, bellied out, perhaps, like the sail of a yacht antero-posteriorly. The joints of the wing are all ginglymoidal, unless indeed a slight lateral flexibility was possible at the closely united compact wrist, which is doubtful; nor could the anterior or radial curvature of the phalanges be much, if any, greater than I have indicated in the restoration. Because of this lack of rotary power of the anterior extremity, I doubt not that the caudal membranous expansion in *Rhamphorhynchus* served as a compensatory steering organ, and the same function was subserved, in a somewhat different way, by the legs in the short-tailed forms. But there are much better reasons for supposing that the wing membranes continued to the legs or ankles of these animals. The peculiar structure and evident position of the legs would have been inexplicable under any other assumption, but if the membrane was restricted to the sides of the body the patagial surface must have been a mere ribbon, five or six inches wide and nearly four feet long, scarcely serviceable as a wing or parachute even!

If the animals stood erect when upon the ground, with the knees rotated outward and the tibiæ parallel, the wings, drooped at the side, which may have been possible, would have reached the surface at the metacarpophalangeal joint, with the phalanges trailing or partly folded; and it is possible that in such a position, partly stooping, partly creeping, the creature might have laboriously got about on land. But I do not think that they often voluntarily sought the ground for ambulation. Their home was in the air, and they rested suspended by the flexible, sharply-clawed middle fingers. The elongated zygapophyses of the first dorsal vertebra, a functional cervical, though structurally a dorsal, indicate, not a marked backward curvature of the neck at this place, but rather the possibility of a marked anterior curvature. Perhaps the neck was sufficiently flexible to permit a strong sigmoid curvature, bringing the head in a forward direction when in a prone position.

It is commonly believed that the small, slender bone articulating with the wrist and directed backward toward the shoulder—the so-called pteroid or thumb metacarpal—was for the support of the membrane in front of the elbow.

I am satisfied that this was not its function, for the simple reason, as I believe, that there was no membrane there, unless it extended on the sides of the neck broadly towards the skull! This may seem a mere

assumption, but there are evidences in its favor that give this idea some weight. The strongly developed deltoid process shows attachments for several muscles. One occupied the whole distal anterior face, as indicated by an oblique line running from near the distal lower extremity inward and upward. This was doubtless for the insertion of the supracoracoid muscle, the origin of which is shown by a strong tuberosity on the upper part of the coracoid, and whose tendon was guarded in part, apparently, by a sesamoid bone found near the glenoid articulation. On the distal convex border, near its upper extremity, there is a small facet for muscular attachment, looking outward and forward in the extended position of the arm. This may have been for the attachment of the pectoralis, as Plieninger seems to believe, but of which I am very doubtful. Certainly the pectoralis must have been a very weak muscle to have terminated in so small a tendon, and the extensive surface of the sternum calls for a large one. It would furthermore have acted as a powerful muscle of inward rotation, of which the joint was incapable. Lying in front of the arm and adjacent thereto, there are a number of long, thin, striated ossified tendons, with somewhat fimbriated extremities, some of them eighty millimeters in length by four or five in width. I am satisfied that the most of the space in front of the elbow was filled during life by strong muscles, controlling the movement of the arm and wrist, the anterior brachial and carpal flexors, whose origins were high up on the humerus. On the upper distal extremity of the radius there is an articular surface extending backward, and, lying near it in the specimen, there is a small sesamoid bone, doubtless belonging to a carpal flexor. The pteroid bone has a rounded convex articular surface on one side of its broad carpal extremity that evidently fitted into a depression in the lateral carpal bone lying near it. The joint seems to have permitted considerable enarthrodial movement, with but little gliding motion; it clearly permitted considerable oscillation of its free extremity in the plane of the wing. The distal extremity reached nearly to the deltoid process in the ordinary flight position of the wing. There certainly was not sufficient membrane in front of the elbow to need such an elaborate structure for its support if the membrane ceased at the shoulder. On the assumption that this bone is a reversed thumb metacarpal, an altogether probable theory, one cannot conceive how it could have assumed its present position, unless it had been reversed and brought toward the shoulder by the action of a membrane that originally extended along the sides of the body over the outstretched fingers like that of a bat. By the gradual development of the little finger, as is shown, indeed, by the more elongated metacarpal of the

later forms and the greater proportional development of the middle fingers in the early forms, the end of the thumb was drawn backward and rendered tense, until finally its position became directly opposite to the original one.

We must assume then that the membrane originally was developed to a greater or less extent in front of the arm as well as behind it. Had the membrane finally disappeared here, it is only natural to suppose that the bone controlling it would become vestigial—assuming Lamarckian views! On the contrary, it has evidently increased in size, for it seems to be larger in this one of the most specialized of all pterodactyls than in the earlier ones. What then could be its functions, unless as the support of a membrane that extended over the shoulder to the sides of the neck? I can not say that I am convinced that this really was the case in our *Nyctodactylus*, but I think it not improbable.

The attachment of what I believe to be the pectoral muscles was by a stout and prominent process on the inner proximal side of the humerus.[3]

In an earlier communication I stated that the upper part of the bill or beak in Ornithostoma was not produced in a sharp ridge as Marsh stated, but that it was rounded. Plieninger (l. c.), however, thinks that I was mistaken and that Marsh was right. "Die Medianlinie des Schädels ist im vorderen Theile in eine scharfe Kante ausgezogen, welche allmählich nach hinten, gegen die Nasopræorbital Öffnung hin, in eine sanfte, stumpfe Rundung übergeht; allem Anscheine nach ist die scharfe Kante im vordersten Theile nicht durch Druck hervorgerufen, sondern war ursprünglich vorhanden." In the present specimen the skull is in a most admirable state of preservation, and an examination of it proves beyond shadow of doubt that the median part above was roundly, evenly and smoothly convex, at least as far back as the narial opening. "Die Oberfläche der Schädelknochen ist fast durchwegs mit verschieden geformten, meist annähernd ovalen Grübchen bedeckt. Williston glaubt, dass nur der Abdruck der in der spongiosen Masse befindlichen Höhlraume sei, eine Ansicht, welche ich nicht theilen kann."—Plieninger (l. c.). The upper surface of the beak does not show the slightest indications of such depressions, but is perfectly smooth and plane. This condition I have seen so often in *Ornithostoma* that I feel sure that the

[3] Plieninger rightly objects to the use by me of the term "bicipital crest" for this process, by saying that it was not for the attachment of the biceps muscle, which never arises from the humerus. That so unpardonable an error may not be attributed to one who has taught human anatomy for many years, I may say that I used the term, inadvertently, in the anthropotomic sense of the "anterior bicipital ridge" and as such I believe it to be correct, though not a proper term here.

depressions were always the result of the compression to which the bones were subjected.

At present our knowledge of the skeleton of *Nyctodactylus* is nearly complete, more complete perhaps than that of any other pterodactyl known.

Its structure demonstrates the comparative unimportance of the scapular articulation as a diagnostic or classificatory character. The structure of the skeleton throughout, even of the notarium or consolidated dorsal vertebræ, is very much like that of *Ornithostoma* save in the scapula. From this it follows that the genus must be placed in the same family with *Ornithostoma* and *Ornithocheirus*. In my own opinion, there is not even a subfamily difference.

I still believe that the genus *Pteranodon* is identical with *Ornithostoma*, and that the former term must be abandoned. Plieninger (l. c.), however, concludes that even if the two terms be synonymous, the name *Ornithostoma* has no claims for recognition, because it was not adequately described or figured before Marsh described *Pteranodon*. Were this true, and it may be, it would not be sufficient justification for the rejection of *Ornithostoma*. Were the rule applied to Marsh's own names, a large part of them would be rejected, as he rarely gave characters substantiating his terms. But there is a far weightier reason for the abandonment of the name *Pteranodon*. Prof. Seeley, according to his statement,[4] pointed out to Prof. Marsh the toothless character of *Ornithostoma* and showed him the evidence before *Pteranodon* was known!

On every principle of nomenclature and justice the name *Ornithostoma* must take precedence over *Pteranodon* if these genera are found to be identical, as I believe will be the case.

Note.—Since the foregoing has been in type, the skull of the specimen described has been nearly wholly freed from the matrix. It has no occipital crest, and the occiput is a little less produced than in the figure; otherwise the outline is nearly correct. The fossil skull, thirty-one centimeters in length, inclusive of the mandible, weighs less than thirty-nine grammes!

[4] Dragons of the Air. London, 1901. p. 182.

ORIGIN AND MIGRATION OF THE GERM-CELLS IN ACANTHIAS.

BY

FREDERICK ADAMS WOODS, M. D.

From the Embryological Laboratory of the Harvard Medical School.

WITH 14 TEXT FIGURES.

The dog-fish having been used so extensively as a basis for our knowledge of the morphology and development of the genito-urinary system, any further contribution in this department of anatomy can be easily fitted in to larger accounts, such as we have from the well-known investigations of Balfour, **78**, and Semper, **75**, who used this and closely allied species in their pioneer genito-urinary researches.

"Since Semper's time, in 1875," to quote from Dr. Minot's Embryology, p. 250, "it has come to be more and more generally admitted that the development of the genital glands leads in both sexes through an early stage characterized by the appearance of primitive ova (*Ureier*, Primordialeier, ovoblast). The primitive ova are merely enlarged cells of the germinal epithelium (or so-called medullary cords)."

Balfour gives the following location of primitive ova when first observed, **78**:

"The primitive ova are confined to the region which extends posteriorly nearly to the end of the small intestine and anteriorly to the abdominal opening of the segmental duct.

"The portion of the mesentery in which the primitive ova are most densely aggregated corresponds to the future position of the genital ridge, but the other positions occupied by the ova are quite outside this. Some ova are in fact situated on the outside of the segmental duct and segmental tubes, and must, therefore, effect a considerable migration before reaching their final positions in the genital ridge on the inner side of the segmental duct."

These cells destined to form the ova in the female and probably the spermatozoa in the male are to-day generally considered to be derived from the germinal epithelium of the genital gland. The epithelium itself is a modification of the embryonic peritoneum and its special region is indicated in the drawing of the 19 mm. embryo (Fig. 14 Ur).

Vol. I, Part 1, p. 125, of Quain's Anatomy contains a well-known picture taken from Balfour and is designed to show this transformation of epithelial cells into sex-cells and is labelled "Transverse section through the ovary of an embryo shark showing the germ-epithelium forming primitive ova."

Later investigators in the embryology of this region in the dog-fish, shark, etc., have also ascribed the origin of these cells to transformation of the cœlom epithelium, and have attempted to account for their presence in the unusual and distant positions.

Ruckert, 88, found the primitive ova in the segmental mesoderm of Selachieme and made the observation that only a few of the cells of this type lie outside in the unsegmented mesoderm, thus giving support to his belief in the Gonotome theory—that is, that the reproductive organs of the vertebrates were originally segmented like the vertebræ themselves.

This use of the word Gonotome was called in question by Minot in 1894, in an article "Gegen das Gonotome," claiming that our knowledge about these large cells was not sufficient to warrant us in believing them to be necessarily all primitive ova since some were in positions entirely outside the genital region. As we had no exact knowledge of the origin, fate or meaning, they might even be ordinary cells in the process of division.

Carl Rabl, 96, believed these peculiar cells to be all primitive ova. He found them first over a diffuse region lining the body cavity which region subsequently became contracted. They were situated in both the splanchnopleure and somatopleure, though most of them were in the former.

Like Balfour he could not explain their peculiar structure or the granules of yolk in their protoplasm. He considered it difficult to explain their disappearance from the somatopleure since there was no certain evidence of their migration, and he also suggested the idea that these more distant ones might be changed over into ordinary epithelial cells. He did not hint at their presence before the formation of the cœlom. Thus the classical view is, that the germ-cells originate in the cœlom epithelium.

More recently, a few researches regarding the origin of the primitive ova in a few more or less peculiar lower vertebrates show that in certain forms at any rate these cells are differentiated very precociously even before any embryo is formed and never arise from any somatic or body cells.

My own investigation on the origin of the primitive ova in Squalus acanthias or the common dog-fish, a typical elasmobranch, entirely dis-

credits the view that these cells arise from epithelium and shows that the germ-cells are traceable out of the mesoderm as we seek them in younger and younger embryos, until their origin is placed in the endoderm or underlying yolk, even before any mesoderm is formed.

In fact, as far as determined, from their first appearance up to the time of their situation in the genital region when they become the acknowledged primitive ova, these cells do not appear to be differentiated at all.

It is the other or somatic cells which become changed during the processes of growth.

In the youngest embryo studied, in the blastoderm stage, practically all the cells, except those of the ectoderm have all the characteristics of the primitive ova found in later embryos of from one to six millimeters.

It will be seen from the drawings that while most of the cells of the endoderm and mesoderm are losing their clear, round outline, and also their yolk, fusing together into a net-work, or being changed into tall, columnar epithelium, some cells retain all their original characteristics.

It will also be seen that at least some, if not all of these cells of the original type are gathered up in a small tumor-like ball at the junction of the endoderm and mesoderm before the mesoderm has split, and that afterwards these spread through this layer and by actual migration find more and more their positions in the segmented mesoderm until they finally rest exclusively in the genital region of this germ layer. During no part of this migration have any cells been seen that would lead one to suspect a transition of the mesoderm or mesothelial cells into primitive ova.

After the mesoderm is once formed and the ova are once in it, there is no difficulty in determining whether any given cell is or is not a germ-cell.

Although several thousand were counted with oil immersion, no doubtful cells were met.

The ordinary cells of the mesoderm give no clear cell outlines, they fuse together and are practically devoid of yolk. Such shapes as are indicated are small spindles and cylinders. The primitive ova are large oval or spherical, have clear cut cell walls, and are crowded with yolk. A peculiar notching of the nucleus is often present and has been suggested as characteristic of these cells, but as it is found in cells which do not in other ways conform to this type and as it does not appear to be present in all of them, I do not feel that this is a sure test.

Of course the youngest embryos, i. e., those without a mesoderm,

give difficulty since the younger the embryo the more the cells in general are filled with yolk spherules and conform in other ways to the germ-cell type.

Let us follow the changes onward from the young blastoderm up to the point of sexual differentiation of the genital gland in embryos of 34 mm.

The first cut (Fig. 1) is taken through the greatest diameter of an embryo of Balfour's stage A, at the growing point. The cells of the ectoderm are somewhat differentiated, being long and slender, the other

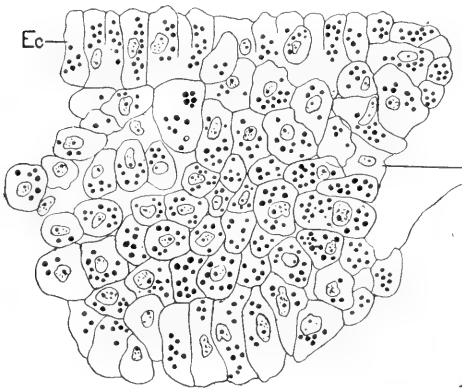


Fig. 1.

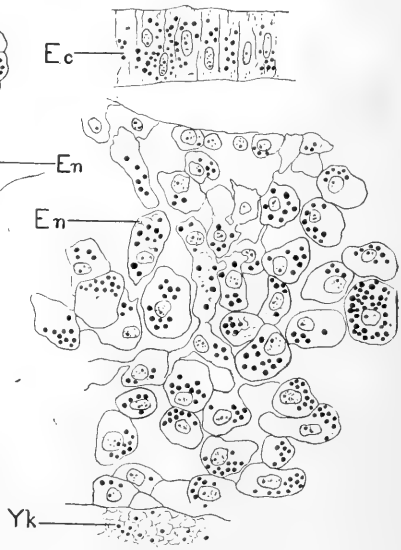


Fig. 2.

Fig. 1. Blastoderm, Balfour's Stage A, at the growing point. Ec. ectoderm, En. endoderm. Harvard Embryological Collection, Sag. Series, 490, section 81. × 330.
Fig. 2. Blastoderm, slightly older than Balfour's stage A. Ec. ectoderm, En. endoderm, Yk. yolk. × 330.

or endodermal cells are nearly all alike and of such characteristics that found in the mesoderm of a dog-fish 3-6 mm., I should unhesitatingly call them primitive ova. No mesoderm is yet distinguishable.

The second section (Fig. 2) is slightly older, still no mesoderm is differentiated. Many of the cells of the endoderm show indefinite limits in certain directions, long processes of protoplasm tending to join them in a net work. Some of the cells, however, retain the earliest type.

A section of a later age, Balfour's stage D, 2½ mm. long, shows the mesoderm clearly formed on each side of the embryo. The split in the

mesoderm which will form the cœlom has, however, not yet taken place. At this stage most of the mesoderm and endoderm cells have altered their original characteristics, being smaller and less clear in outline, taking on spindle or cylinder shapes, losing more or less of the darkly-stained yolk granules and tending to fuse into a more mesenchyma-like mass.

Here and there, however, in the extra-embryonic mesoderm, endoderm and even in the yolk just beneath the endoderm, one finds numerous cells of the original type.

The appearance of such cells in the yolk just beneath the endoderm and even frequently partly in the endoderm and partly in the yolk suggests the idea that cells of the early type are being formed in the yolk itself at the period during which the embryo undergoes its first two or three millimeters of growth and that these cells contribute to the formation of the endoderm and perhaps also to the *Ureier*. This contribution of yolk cells to the endoderm is stated in Balfour. Or it may be that the *Ureier* are derived solely from such yolk-formed cells. This would give the *Ureier* an extra embryonic formation similar to the blood corpuscles as claimed by His. "Angioblast," oo.

Whether the *Ureier* are formed in the yolk or in the endoderm, cells of this type are now present in both and also in the mesoderm, but only near its junction with the endoderm.

It is important that so far none of the cells in the segmented mesoderm or even near the segmented mesoderm have any such characteristics. All cells of regions are of the small, dimly outlined variety, carrying only a few yolk granules.

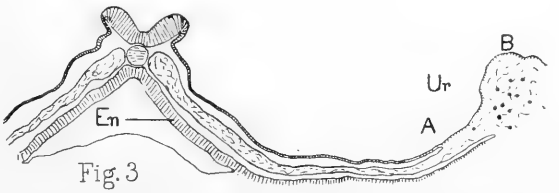


Fig. 3

FIG. 3. Cross section, embryo of 2¾ mm. En. endoderm, Ur., germ-cells. A-B., location of all the germ-cells. Harvard Embryological Collection. Trans. Series 462, section 90. × 38.

The next section (Fig. 3) is taken through the hind segment of a 2¾ mm. embryo with 9 somites. This is through the heart of the primitive ova region and shows that these cells are found far out on the yolk in the early stages. It is possible to count them at this stage since all the remaining cells have changed their characteristics in one way or another. There were 93 of them present on the right side and all lay within the region marked A-B, most of them being in the noticeable swelling caused by the union of the three germ layers. Only 5 were in the mesoderm.

During these early stages from 9 up to 30 somites more or less, the primitive ova always lie at the hind end of the embryo where the germ layers meet or are also a little anterior to this junction. In the youngest embryo before the medullary groove has closed, they lie quite far out on the sides and always in the blastodermic rim.

Fig. 4 is from a 3 mm. embryo. The germ-cells are found near the junction of the three germ layers and are nearer the median line than in Fig. 3.

Fig. 4. Embryo 3 mm. En. endoderm, germ-cells as black dots. × 38.
Fig. 5. Embryo 3½ mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 463, section 147. × 38.

The next section, Fig. 5, is from an embryo of 3½ mm. This shows the primitive ova at the junction of the mesoderm and endoderm, huddled together in a characteristic tumor-like ball as they lie before their journey upwards into the segmented mesoderm and genital region.

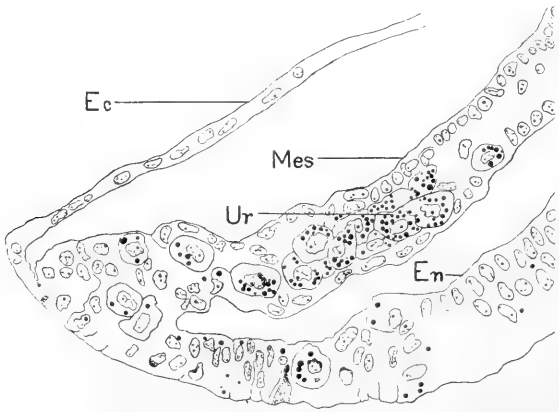


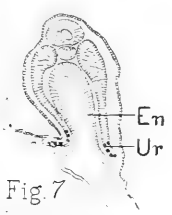
Fig. 6. Section of germ-cell region drawn with oil-immersion objective. Embryo of 3½ mm. Ec. ectoderm, En. endoderm, Mes. mesoderm, Ur. germ-cells. × 330.

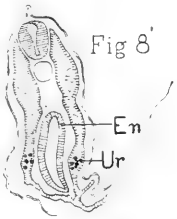
This cluster is where most of the primitive ova lie and is just anterior to the last body segment where the three germ layers come in contact. A little mass like this is found on both sides of the body in all embryos between about 3 and 4 mm., and is so conspicuous as to be easily seen

with low powers of the microscope and should be included in a description of the gross anatomy of the part. As yet there are no cells of this type higher up in the mesoderm.

The above section, Fig. 6, was taken at the junction of the three germ layers in the posterior region of another embryo of 3½ mm. It will be seen that the ball cluster is broken up and also that the cells are found at some little distance up into the mesoderm. On one side in this embryo there were 230 cells of the primitive ova type, all very near together in this part of the body.

Another section, Fig. 7, shows the position of most of the *Ureier* in a 4 mm. embryo. These clusters of primitive ova were mentioned by Balfour who observed them in the genital regions of older embryos and considered them due to rapid cell division. I have never seen, during these early stages, any indications of cell division. It would seem that





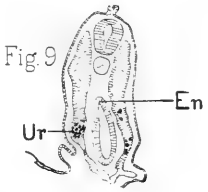


Fig. 7. Cross section embryo 4 mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 464, section 123. × 38.

Fig. 8. Embryo 5 mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 231, Section 275. × 38.

Fig. 9. Embryo 6 mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 293, section 318. × 38.

these clusters or egg nests were here merely due to the fact that a little earlier, they practically all lie in two groups, one on each side in the narrow mesoderm and that in such a drawing as Fig. 8 they have not yet all wandered away from each other.

Fig. 8 shows the principal location of the cells in a 5 mm. embryo. Fig. 9 is of a 6 mm. embryo. One cell on the right is indicated as having reached the segmented mesoderm. Up to this point the mesoderm has not split. The next section, Fig. 11, embryo of 8 mm. shows the split in the mesoderm to form the body cavity. The primitive ova practically all succeed in getting on the splanchnopleure or inner layer; just how is, to me, a mystery.

A few are now in the genital region or dorsal end of the cœlom, but most of them are in the epithelium all along around the intestine and mesentery.

The larger mass of cells on the right shows the region in which most of the sex cells are scattered. It will be observed that even yet the genital fold has not formed. There are in this embryo 154 primitive ova in the region ventral to the mesentery. Seventy-three are in the mesentery and 69 in the genital region.

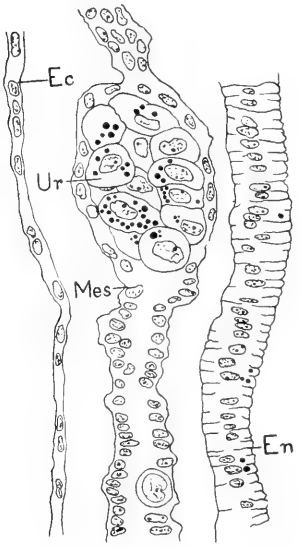


FIG. 10. Germ cells as they appear in a 6 mm. embryo as Fig. 9. Ec. ectoderm, En. endoderm, Mes. mesoderm, Ur. germ-cells. × 330.

Fig. 12 is a cross-section through an 11.5 mm. embryo. It shows the greatest height of the embryo above the yolk and also the corresponding length of the intestine. The black dots indicate the position of the primitive ova and the relatively large space that they now cover. About half of them would be found in the mesentery at this stage; 137 were counted in this structure.

Fig. 13 is a cross-section through a 15 mm. embryo at a point a little posterior to the last in a region where the connection of the embryo with the yolk does not show. In such an embryo most of the germ-cells are in or near their ultimate position in the thickened epithelium near the letter d. The great accumulation in the mesentery has been transferred to the posterior cœlom epithelium. There were 29 in the mesentery and 41 ventral to this.

Fig. 14 shows a cross-section of a 19 mm. embryo. At this stage most of the cells have reached the region of the genital fold. Many, however, are at the root or posterior portion of the mesentery, and a few are still in the mesentery itself.

Out of 272 primitive ova, 242 were in the genital fold now shown on the left or just to the median side of it. There were 19 in the mesentery and 11 ventral to the mesentery.

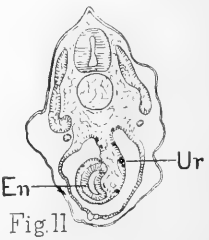


Fig.11

FIG. 11. Embryo of 8 mm. Most of the germ-cells are near the point Ur. En. endoderm. Harvard Embryological Collection. Trans. Series 447. × 38.

In an embryo of 28 mm. we see the genital gland formed, and of 473 cells present, 469 were completely housed in the gland itself. The remaining four were close at hand, being in the root of the mesentery. An embryo of 34 mm. was

also used for a count; there were 710 germ-cells and all were in the genital glands.

The following table shows the proportionate distribution of primitive ova from the time when they could first be distinguished up to the complete formation of the genital gland at about the time of sexual differentiation into male and female.

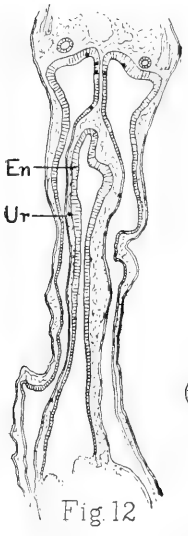


Fig. 12

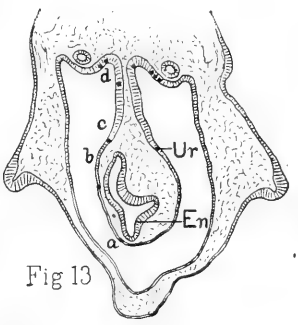


Fig 13

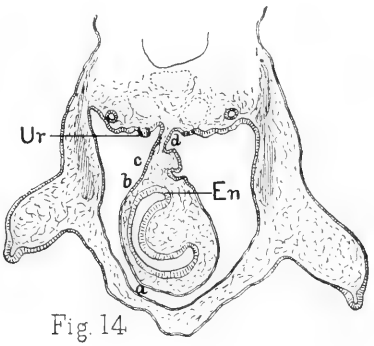


Fig. 14

Fig. 12. Cross-section through the gut region of an embryo of 11.5 mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 206.

Fig. 13. Ventral portion of an embryo of 15 mm. En. endoderm, Ur. germ-cells. Harvard Embryological Collection. Trans. Series 499, Section 227. × 38.

Fig. 14. Ventral portion of an embryo of 19 mm. En. endoderm, Ur. germ-cells in the genital gland. Harvard Embryological Collection. Trans. Series 137, section 390. × 38.

Length of Embryo in mm.	Total No. of Ova.	Unsegmented mesoderm or ventral to the mesentery.	Mesentery, when formed.	Segmented mesoderm. Later genital region.
2.75	98 [1]	98	..	..
3.5	230 [1]	230	..	..
4.5	237	216	..	21
5.	128	110 [2]	..	18
6.	256	222	..	34
8.	296	154	73	69
11.5	408	78	137	193
15.	346	41	29	276
18.	240	17	14	209
19.	272	11	19	242
28.	473	4	..	469
34.	710	..	..	710

Mode of Migration.

There is no question but what these cells migrate with reference to the embryo as a whole since the number ventral to the mesen-

[1] One side only counted.

[2] Part of series lacking.

tery, in the mesentery and finally dorsal to it, shifts by regular transitions and furthermore the number at first close together becomes secondarily scattered over a wide area and then contracted, but the cause of this migration is somewhat difficult to determine.

There are two views regarding this as well as a combination of the two possible.

First, they may migrate by independent amœboid motions through the cells that surround them.

Second, they may migrate relatively to the embryo by themselves, remaining comparatively fixed and many complicated changes in various tissues of the embryo causing a shift of their position.

It is easy to conceive how a growing together of the two lateral lips of the blastodermic rim might convert Fig. 3 into Fig. 5, how a growth of somatic cells into the ball together with an elongation and narrowing of the embryo as a whole might bring about Fig. 7.

Fig. 9 might be formed by a sinking of the intestine with reference to the mesoderm combined with a segmenting of the mesoderm in a more and more ventral direction, thus bringing some ova to lie in the segmented mesoderm though none lay here in Fig. 7.

In comparing the Figs. 7 and 9, it does not seem impossible that this may be caused by a growth of tissue between the notochord and endoderm, together with a contracting down of the endoderm into a smaller circumference.

This would mean that the region between the primitive ova and the top of the protovertebræ has not grown any in length from the condition in Fig. 5, where we see that the ova nearest to the top of the protovertebræ are fully as far away from the top as they are in Fig. 9 on the right, or even about as far as they are in Fig. 11.

It is to be remembered that Figs. 3-5, 7-9, 11-14 are all drawn with a camera lucida with the same magnification so that the drawings relative to each other represent the actual relative sizes of the embryos though all are enlarged.

The embryo during these changes has grown but little in height from Fig. 3 to Fig. 11. It can easily be conceived how an unfolding of the two lateral parts of the embryo in Fig. 5, combined with a splitting of the mesoderm, with a sinking of the gut might produce Fig. 11, adding, of course, certain other changes in the sizes of parts like the enlargement of the notochord, spinal cord, etc.

We can reconcile Fig. 11, 8 mm., with Fig. 13, 15 mm., and not introduce the question of independent amœboid motion, if we suppose that the mesothelium of the cœlom ventral to the mesentery and surrounding

the gut migrates as a whole over the surface of the mesenchyma or that the gut, as a whole, sinks further and growth of tissue takes place in its lower parts.

However, there comes a stage now which seems to me incompatible with any view except that of independent motion through the tissue. This is the transition between Fig. 13, 15 mm., and Fig. 14, 19 mm.

In the 15 mm. embryo there were 276 primitive ova in the posterior epithelium of the cœlom, 29 in the mesentery and 41 ventral to this point. Of these 41 occupying ventral positions there were 28 which are especially interesting in trying to prove this point since these lie around the intestine and even directly under it. Nineteen of these 28 lie in the territory between a and b and 9 lie between b and c.

In the 19 mm. embryo there were 242 primitive ova in the genital region, 19 in the mesentery and 11 ventral to this point. Nine of these were in the region b-c. None were in a-b, though the entire embryo was searched. The other 2 lay much further away, near the remains of the yolk. This was confirmed by a study of an 18 mm. embryo.

These changes between the 15 and 19 mm. are entirely compatible with migration through the tissues and it seems to me with no other view. It is not conceivable or at least extremely improbable that any special growth should raise cells from point a to point c. The only other alternative would be degeneration in situ of the cells between a-b in the 15 mm. embryo.

I have looked for such degeneration in the cells under the alimentary tract, but have never observed any such evidences. Whenever they were found they have always, with very few exceptions, shown out clearly and with no transitions wherever their positions may have been. So it seems that the changes in the early stages are due mostly to changes in relative growths of the different parts of the embryo and that in the later stages at any rate we must add an independent migration through the epithelial cells. This independent migration, though remarkable and difficult to explain, is somewhat paralleled by young nerve cells whose movements through tissue are acknowledged to take place. It is very hard to understand how these cells can migrate even for a little distance through the cells of a columnar or low cuboidal epithelium and practically never fall out or wander into the underlying tissue. Yet such appears to be the case, especially in the stages between 11 and 19 mm.

It may be that some chemical forces, which we at present in our ignorance call chemotaxis, are factors taking an important part in the development of tissues.

It is interesting to note that just before the *Ureier* are taken into the mesoderm they all lie near the junction of the three germ layers. It may be that reproductive cells which have as they do, possibilities for formation of all three layers can come only from a region in the embryo in which the somatic cells themselves have possibilities in one of these three directions.

With regard to its bearing on the theory of the gonotome, the evidence is, of course, opposed to such a theory.

As we trace to its early condition, the sexual gland, we do not find it originally segmented. On the other hand, it is first unsegmented and subsequently becomes segmented, because it must, since it moves into the body cavity which is itself segmented.

Summary.

To summarize: The most important conclusion appears to be that the germ-cells in the dog-fish are not developed from somewhat specialized cells of the body, but that a few undifferentiated cells of the earliest type are taken out and passed on until the new individual is formed.

This early appearance of germ-cells in vertebrates is not a new discovery, since it has been observed by Eigenmann in Cymatogasta, 91, a bony fish. Eigenmann, however, considers this fact to be in some way associated with peculiarities of other sorts in this fish.

J. Beard, 00, has announced the early appearance of the germ-cells in Raja batis, the skate, though his paper containing the proofs is still wanting.

In the lamprey, according to W. M. Wheeler, 99, the germ-cells are not derived from the mesothelium cells, but appear very young in the endoderm.

In all these forms cited above, the germ-cells had not been studied at all prior to these investigations, so it was not so much that the epithelial origin was overthrown as that such origin was not the true one in these special cases.

The value of finding a similar condition in the dog-fish and shark seems to me to be twofold—first, because these have long been used so much by investigators and students as typical lower vertebrates, and second, it shows that even among them where an epithelial origin was contended the early endoderm origin is likewise true, thus bringing them all in the same category.

Lastly, its bearing on the continuity of the germ plasm. Moritz Naussbaum, in 1880, formulated an hypothesis that "the sexual cells

do not come from any cells that have given up their embryonic character or gone into building any part of the body, nor do sexual cells ever go into body formation." This he considered only an hypothesis without much basis of observation.

This hypothesis was taken up by Weismann, in 1883, who, like Naussbaum, insisted that such was inconsistent with Darwin's hypothesis of pangenesis and that the reason why the offspring is like the parent is because some germinal cells are saved out unchanged.

As Francis Galton expressed it, the individual is merely the "trustee" for the cells that maintain the species.

It seems to me that the facts about the primitive ova in the dog-fish amply bear out the hypothesis of Naussbaum, while regarding Weismann's later contentions concerning the inheritance of acquired characteristics, even if such early origin of germ-cells were proved by finer methods to be the condition in the higher vertebrates as well, his contention would not be proved, though as far as it went, it would be strengthened since there would be more reason still for looking upon the primitive ova and the offspring as well, as collateral with, rather than a part of the parent.[3]

Finally, I wish to express my obligations to Dr. Charles S. Minot for his many useful suggestions during the progress of this research.

BIBLIOGRAPHY.

Balfour, F. M., 78.1.—Development of Elasmobranch Fishes, Chap. VI.

Beard, J., 00-1.—Morphological continuity of the germ-cells in Raja batis. Anat. Anz., Dec., 1900.

Eigenmann, C. H., 91.1.—Precocious Segregation of the Sex-cells in Micrometrus Aggregatus. Journ. Morph., V, 481-492.

————, 95.2.—The History of the Sex-cells from the time of Segregation to Sexual Differentiation in Cymatogaster. Trans. Amer. Micros. Soc., XVII, 172-173.

Hacker, V., 97.1.—Die Keimbahn von Cyclops. Arch. f. Mikros. Anat., XLIX, 35-91.

His, W., 00.2.—Lecithoblast u. Angioblast der Wirbelthiere. Abhandl. Math. Phys. Classe K. Sachs-ges. Wiss., XXVI, 173-328.

Jungersen, H. F. C., 89.1.—Beiträge zur Kenntniss der Entwickelung der Geschlechtsorgane bei den Knochenfischen. Arbeit. Zool. Zoot. Institut., Würzburg, IX, 89-219.

Minot, C. S., 94.1.—Gegen das Gonotome. Anat. Anz., IX, 210-213.

[3] Beard's latest article, "The Germ Cells of Pristiurus," which appeared (Anat. Anzeiger, xxi, 50) after this article was in type, does not give results wholly in accordance with my own.

RABL, C., 96.2.—Ueber die Entwickelung des Urogenital Systems der Selachier. Morphol. Jahrb., XXIV, 632-767.

RUCKERT, J., 88.1.—Ueber die Entstehung der Exkretionsorgane bei Selachien. Arch. Anat. Physiol., Anat. Abt., 205-278.

SEMPER, C., 75.2.—Das Urogenital System der Plagiostomen und seine Bedeutung für das der übrigen Wirbelthiere. Arb. Zool. Zoot. Inst., Würzburg, II, 195-509.

WALDEYER, W., 70.1.—Eierstock und Ei, 800, Leipzig, 174 p.

WHEELER, W. M., 99.1.—The Development of the Urinogenital organs of the Lamprey. Zool. Jahrb., XIII, 1-88.

THE SPERMATOZOA OF ALLOLOBOPHORA FOETIDA.

BY

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With 1 Plate.

In the spermatozoön of *Allolobophora* we have demonstrated three centrosome-like structures, one at the base of the spine, one at the anterior, and one at the posterior end of the middle-piece. Heretofore we have differentiated these structures at such rare intervals, we could not claim for them any morphological value, but quite recently, by the aid of photography, we have been able to demonstrate these bodies with sufficient constancy to warrant a consideration of their morphological and functional significance. Do these bodies represent merely points of insertion for the spine, middle-piece and tail, comparable to the basal bodies of cilia, or have they a bearing upon the problems of fertilization?

The morphological value of the apical centrosome-like body is enhanced by the fact that a few investigators, Platner (12), Carl Niessing (11), and Field (3), have traced the centrosome of the spermatocytes to the apex of the head of the spermatozoön, and in one case at least, this apical centrosome appears to function in the fertilized egg, as the centrosome of the male attraction-sphere. To this may be added the interesting observations of King (7), who has shown in the egg of *Bufo* that a male aster is formed at the apex of the head of the spermatozoön.

The morphological value of the centrosome-like body in the middle-piece is enhanced by the interpretations of a large number of investigators who have traced the centrosome of the spermatocytes to the middle-piece of the spermatozoön, in many cases this centrosome being identified in the egg, as the centrosome of the male attraction-sphere.

In the middle piece of *Allolobophora*, however, we have demonstrated two centrosome-like bodies instead of one, and in this connection Lenhossék's (8), observations on the spermatogenesis of certain vertebrates are of interest. He identifies two centrosomes in the middle-piece, these having originated by the division of the centrosome of the spermatid. He shows also a centrosome-like body at the apex of the head—his *Akrosoma*—which he claims, however, has no connection with the centro-

some of the spermatid, arising merely as a thickening of the sphere substance. With improved technical methods we hope to be able to identify within the egg the three centrosome-like bodies of the spermatozoön of *Allolobophora* and determine whether any of them function as focal points for astral rays. For the present the only evidence in the egg, indicating that we may expect to find a centrosome in the spine and in the middle-piece is that the cytoplasm of the egg reacts to *both* spine and middle-piece, this reaction being expressed by two morphologically similar structures, the fertilization cone and the sperm aster, these two structures in turn resembling morphologically the asters of the maturation spindles, each of which contains a centrosome.

On several occasions we have called attention to the similarity of the fertilization cone and the male aster, further homologizing these structures to the poles of a spindle. We quote the following from a former paper: "It is impossible to avoid drawing conclusions as to the morphological significance of the resemblance between the male aster and transverse sections through the fertilization cone. The rays and the central aggregation of *Archoplasm* are as pronounced in the one as in the other, suggesting that each end of the head of the sperm—the spine and the middle-piece—produces on the cytoplasm of the egg a like morphological effect. This would indicate that the spine and the middle-piece are of the same substance, though the identity can not be complete, as the cytoplasm does not react to the two structures at the same stage of the development of the egg. . . . The effect produced by the spine is made, however, by a *moving* object (the sperm entering the egg) and we have thus a different shaped aster—a cone shaped aster. Is it possible that this may have any bearing on the opposing interpretations of various authors, some asserting that the anterior end of the head of the sperm produces the male aster, and others, that the posterior end of the head (the middle-piece) produces it?

"If we accept the interpretation of those authors who claim to have traced a part of the aster of the spermatid, to both spine and middle-piece, may we not regard that part of the spermatozoön (including spine, head and middle-piece) as an attenuated spindle, and expect that each end of this spindle will produce a like morphological effect upon the cytoplasm of the egg?" (5) page 605-6.

When the above was written we had not succeeded in establishing the identity of centrosome-like bodies in either spine or middle-piece, though homologizing the spine, head, and middle-piece of the spermatozoön to an attenuated spindle, made this identification very desirable.

If it can be proved that the fertilization cone and male aster are mor-

phologically alike, it would be convenient to designate them as the *anterior* and *posterior male asters*—and it is impossible to resist an attempt to homologize them to similar structures described for other eggs—although we appreciate the danger of making too rigid an application of the phenomena observed in individual cases.

Lillie (10) in his suggestive work on *Unio* describes two asters, the first, which he identifies as the true sperm attraction-sphere, appears and disappears at about the same stage of the egg's development, as the *anterior sperm aster* (the cone) of *Allolobophora;* while the second, his "accessory aster," appears and disappears at about the same period as the *posterior sperm aster* of *Allolobophora.* It is significant, that the sperm aster of *Unio* is "comet shaped," thus resembling the fertilization cone of *Allolobophora,* and it is also significant that it is found, sometimes preceding the head of the sperm. Is it not possible that the male aster and accessory aster of *Unio* correspond to the *anterior* and *posterior sperm asters* of *Allolobophora,* the approximate agreement in the time of their appearance and disappearance being due to the fact that both eggs are fertilized at about the same stage of development?

In *Axolotl,* Fick (2) figures a distinct spherical body at the base of the spine of the spermatozoön, although he does not call it a centrosome. In the egg he sees a cytoplasmic reaction to the head of the spermatozoön, the so-called funnel. Fertilization occurs later in this egg, than is the case in *Allolobophora*—and yet the funnel and the sperm aster of *Axolotl* are undoubtedly homologous to the *anterior* and *posterior sperm asters* of *Allolobophora.*

In the egg of *Allolobophora* fertilization occurs very early, this fact marking the individuality of these two structures, the *anterior male aster* (the cone) appearing at the metaphase of the first maturation spindle, and the *posterior male aster,* after the first polar body is formed —thus separating the two structures by a period of time as well as position. In eggs in which fertilization takes place later, the cytoplasmic reactions to the spine and middle-piece following each other very rapidly, is it not possible that in some cases the *anterior* and *posterior male asters* may be fused or confused?

If we call in evidence the data indicating that division is one of the life expressions of the centrosome, and if we interpret the three small bodies in the spermatozoön of *Allolobophora* as centrosomes, it involves the unauthorized assumption that the centrosome of the spermatid divides, part being destined to the apex of the head and part for the middle-piece of the spermatozoön, these centrosomes being the equivalent of the one centrosome left in the egg after the formation of the

second polar body, and it is an interesting fact that several investigators have observed the division of this centrosome in other eggs.

If, on the contrary, we attribute to these three spermatic structures the value only of basal corpuscles, we still do not escape the centrosome problem, for Lenhossék (9), and Henneguy (6), claim that the basal corpuscles of cilia have their origin in the centrosome.

If they are indeed centrosomes, we must follow their logical implication and admit that they can be placed in evidence for the theory that the centrosome has its stage of activity and its stage of rest, the former represented by the aster, the latter by the so-called naked centrosome. The stage of activity of the spine and middle-piece centrosomes—assuming they are such—has but an ephemeral expression in the egg, and it seems only logical to assume that after this period of activity they may return again to a resting stage. With more exact technical methods, it may be possible to trace them in the egg during the resting stage and this can be assumed also for the egg centrosome. We wish to accentuate these points, as the egg of *Allolobophora* has heretofore given evidence only, in favor of the theory that the centrosomes arise de novo, and are "the expression rather than the cause of cell activity" (4).

This evidence, in brief, is as follows: The complete disappearance of both male and egg attraction-spheres at a definite stage of the egg's development. A lack of decisive evidence that the rays of the male aster focus at any one point in the middle-piece (5), or that the rays of the cone focus at the base of the spine. Further, an inconstancy in both size and form of the egg centrosome at a given stage of the development of the spindle, and a lack of evidence of any division of either egg or sperm aster.

Although the greater part of this evidence is negative, we have no right to ignore it—we may say rather, that the centrosomes of *Allolobophora* present conflicting evidence that demands rigid cross-examination.

BIBLIOGRAPHY.

1. Ballowitz, Emil. Untersuchungen über die Struktur der Spermatozoen, zugleich ein Beitrag zur Lehre vom feineren Bau der kontraktilen Elemente. Zeit. f. wiss. Zool., Bd. 50, Hft. III, 1890.

2. Fick, R. Ueber die Reifung und Befruchtung des Axolotleies. Zeit. für wiss. Zool., Bd. LVI, Hft. IV, 1893.

3. Field, George Wilton. On the Morphology and Physiology of the Echinoderm Spermatozoön. Journ. Morph., Vol. XI, No. 2, 1895.

4. Foot, Katharine. The Origin of the Cleavage Centrosomes. Journ. Morph., Vol. XII, No. 3, 1897.

5. Foot and Strobell. Photographs of the Egg of Allolobophora foetida I. Journ. of Morph., Vol. XVI, No. 3, 1900.

6. Henneguy, L. F. Sur les rapports des cils vibratiles avec les centrosomes. Archives d'anatomie microscopique, Vol. I, 1898, p. 494.

7. King, Helen Dean. The Maturation and Fertilization of the Egg of Bufo lentiginosus. Journ. Morph., Vol. XVII, No. 2, 1901.

8. von Lenhossek, M. Untersuchungen über Spermatogenese. Arch. f. mik. Anat., Bd. 51, Hft. 2, 1898.

9. von Lenhossék, M. Verhandl. der anatomischen Gesellschaft in Kiel. 1898, p. 117.

10. Lillie, Frank R. The organization of the Egg of Unio, based on a study of its Maturation, Fertilization and Cleavage. Journ. Morph., Vol. XVII, No. 2, 1901.

11. Niessing, Carl. Die Betheiligung von Centralkörper und Sphäre am Aufbau des Sämenfadens bei Saugethieren. Arch. f. mik. Anat., Bd. XLVIII, Hft. I, 1896.

12. Platner, Gustav. Beiträge zur Kenntniss der Zelle und ihren Theilung. Arch. f. mik. Anat., Bd. XXXIII, Hft. II, 1889.

EXPLANATION OF PLATE.

The spermatozoa shown in this plate were collected at different times from spermatophores found in the slime tubes removed from copulating worms. In each case one spermatophore was teased in a drop or two of water, spread on a slide and dried in the air, or by heat from an alcohol flame. The preparations were stained at once with iron haematoxylin and mounted in balsam.[1]

For the photos taken at magnifications of 1000 and 660 diameters, a Zeiss apo. 2 mm., immers. lens, 140 apr., was used, with projection ocular 4, (diaphragm at 0) and camera draw demanded for each magnification. For the photos taken at 450 diameters, the Zeiss apo. 4 mm., lens was used, with projection ocular as above.

For convenience we shall designate the three centrosome-like bodies, as the apical granule, and the anterior and posterior granules of the middle-piece. These granules are seen also in spermatozoa found in the spermathecae, but the photo of these was overlooked in preparing the plate.

In the half-tone plate some of the granules were strengthened slightly in order to secure satisfactory printing. If any of our readers should wish to compare the reproduction with the original prints, the latter may be obtained on request.

Photo 1. Spermatozoön, showing spine, head, middle-piece, part of the tail, and the three granules, one between the spine and head, one between the head and middle-piece, and one between the middle-piece and tail. Mag. 1000.

[1] Some of the slides were examined unstained, in glycerine, after five hours' immersion in a saturate solution of osmic acid. The spermatozoa failed to show any osmophile granules.

Photo 2. Spermatozoön from the same spermatophore, though not the same slide as Photo 1. The magnification is less, *i. e.*, 660. The two granules in the middle-piece were not clearly differentiated in this preparation, and we therefore focused on the apical granule. The apical granule is more constantly differentiated than the granules in the middle-piece which require a magnification of one thousand diameters for satisfactory illustration. Photos 3, 4 and 5 further demonstrate this point.

Photo 3. See under Photo 2. Mag. 660.

Photos 4 and 5. Spermatozoa from two spermatophores collected five days apart. Mag. 450. These preparations were photographed to show the constancy of the presence of the apical granule. The lower magnification was used to bring into the field a larger number of spermatozoa than is possible with a 2 mm. lens.

Photo 6. Anterior end of a spermatozoön from the same spermatophore as the spermatozoön shown in Photo 1. On every slide dried in the air, or by heat, there are areas in which entire spermatozoa, or definite parts of them, are much flattened, sometimes the chromatin of the head flowing into a broad, thin layer, in some cases with a line of alveolar cytoplasm on each side of the head, and sometimes the tail splitting into parallel fibres. This is due perhaps to rapid and uneven drying. In such spermatozoa, the three granules are much more clearly differentiated. We have selected a few preparations to illustrate this. In Photo 6, the chromatin of the anterior part of the head is flattened, as described above, and the apical granule sharply differentiated. The effect on the form of the head, produced by the flattening, is seen by comparing the part of the head next the spine, with the part cut by the edge of the photo. Mag. 1000.

Photo 7. Anterior end of a spermatozoön, showing spine and part of head. The head is much flattened and almost completely severed from the apical granule, which is thus sharply differentiated. Mag. 1000.

Photo 8. Spermatozoön showing spine, apical granule and head, the middle-piece with anterior and posterior granules and a part of the tail. The part of the head next the middle-piece is slightly flattened and the tail also is flattened and split into parallel fibres; this condition of the head and tail allowing a sharp differentiation of the two granules in the middle-piece. Mag. 1000.

Photo 9. Spermatozoön with flattened head, showing apical granule and the two granules of the middle-piece. C. f. Photo 6. Mag. 1000.

Photo 10. Spermatozoön, showing spine, apical granule, head with anterior half slightly flattened, and part of the tail. Mag. 1000.

Photo 11. Part of the head of a spermatozoön much flattened; middle-piece showing anterior and posterior granules, and part of the tail split into parallel fibres. Mag. 1000.

The spine with apical granule was not included in this photo, because not on the same plane with the middle-piece, and thus requiring a different focus.

Photo 12. This spermatozoön was photographed to show the fibrillar structure of the tail, this splitting of the cytoplasm of the tail being due

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probably, as stated above, to some unusual condition in the drying on the slide, for it occurs only in definite areas. Mag. 660. The head of the sperm is slightly out of focus, for it was necessary to sacrifice this detail, to get an exact focus on the extremely fine fibrillae of the tail.

This preparation resembles many of the figures Ballowitz gives of the spermatozoa of Coleoptera (1).

ON THE DEVELOPMENT OF THE CONNECTIVE TISSUES FROM THE CONNECTIVE-TISSUE SYNCYTIUM.

BY

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WITH 18 TEXT FIGURES.

It is much disputed whether the connective-tissue fibrils arise within cells or from a substance between them. It matters little which view is entertained, the evidence in either case is unsatisfactory. The many researches upon the development of the connective tissues have not given results fully satisfactory and the reason for this is only too evident to those who have made this subject a special study. To be sure material is very abundant and at first sight the problem is a simple one to be solved easily.

The first marked step in advance regarding the histogenesis of connective-tissue fibrils was made by Flemming in 1891,[1] to be followed by a second communication in 1897.[2] According to Flemming, the fibrils of white fibrous tissue are formed in the protoplasm at the periphery of the cell, then gradually thrown off, after which they may still continue to grow. Simple as this is, it is extremely difficult to prove; for with this problem many others are associated to cause complications.

While there are a number of investigators who support the view of Flemming, there are also a number who oppose it. One of the most recent is Merkel,[3] whose studies were upon the human umbilical cord. Merkel comes to the conclusion that the white fibers are formed in the intercellular substance, as taught by Kölliker. It is unnecessary to enter more into the literature of this subject, for it would only result in arranging the authorities into one group or another, or into an indefinite group. The literature has been collected recently in the article

[1] Flemming, Virchow's Festschrift, Berlin, 1891.

[2] Flemming, Archiv für Anatomie, 1897.

[3] Merkel, Verhandl. d. Anatom. Gesellschaft, 1895.

by Spuler,[4] and the reader interested in this side of the question is referred to it.

My own studies upon the development of connective-tissue fibrils began a number of years ago and my first results were published in 1891.[5] Up to that time I could make but little headway with sections prepared in the ordinary way, and was compelled to use frozen sections and chemicals to analyze them. By using these methods alone I think all observers will also come to the conclusion I did at the time—that the connective-tissue fibrils are intercellular in their formation. Since that time, however, methods have been improved and I have gradually learned that the development of white fibrous tissue is better studied in the skin and superficial fascia of the embryo than in tendons, and that elastic tissue is better studied in the arteries and in elastic cartilage than in the ligamentum nuchæ. I also have found that in their development the reticulum of the liver, the connective tissue of the cornea and cartilage are practically identical with that of white fibrous tissue. Very recently Dr. Sabin, Fellow in Anatomy at the Johns Hopkins University, has followed successfully the development of the reticulum of the lymphatic node. While my results are now decidedly in favor of Flemming's view, the reader will soon see that if other methods and interpretations are employed (which I now consider false), it will be quite as easy to see the fibrils developing between the cells as within them.

This all brings me to a turning point, no doubt the key to the situation. The network of fibrils which forms Wharton's tissue, to employ the best known example, is composed of a mass of anastomosing cells, a syncytium, from which the connective tissues arise. Often this syncytium is very sharply defined and differentiated, with nuclei and a little protoplasm which is less differentiated lying upon it. When differentiated to so great an extent it is very easy to designate the main portion of the syncytium as intercellular in position as well as in origin; and since the connective-tissue fibrils arise directly from it they are of course intercellular in origin. When studying these structures in frozen sections it is quite easy to remove the nuclei, leaving only the fibrils of the syncytium. With improved methods, however, it can be shown that in later stages of the development of the syncytium the nuclei lie upon it and are therefore easily removed. In the earlier stages the nuclei lie within the syncytium, but at this time it is too delicate to isolate by the freezing method.

[4] Spuler, Anatom. Hefte, Bd. 7, 1896.

[5] Mall, Abhandl. d. K. S. Gesellsch. d. Wiss., Bd. 17, 1891.

In very early embryos the mesenchyme is composed of individual cells which increase rapidly in protoplasm and then unite to form a dense syncytium. The protoplasm of the syncytium grows more rapidly than the nuclei divide, so that in a short time we have an extensive syncytium with a relatively small number of nuclei. In its form the syncytium appears as large bands of protoplasm with spaces between them filled at times with cells and at other times with fluid. The second condition we have in the umbilical cord of young human embryos. About this time the protoplasm of the syncytium differentiates into a fibrillar part, which forms the main portion of the syncytium—the exoplasm—and a granular part, which surrounds the nucleus—the endoplasm. The fibrils of the exoplasm are very delicate and anastomose freely. When cartilage develops the exoplasm of the syncytium becomes denser and denser; the nuclei and endoplasm wander into the spaces of the exoplasm, which finally becomes semi-hyaline and takes the characteristic stain of the ground substance of cartilage. Reticulum of the lymph node is easier studied, for here we have the least differentiated form, although the pictures are not so striking as they are in the development of cartilage. The development of the cornea is intermediate between reticulum and white fibrous tissue. In the development of membranous bone the process is similar to that of cartilage, only that the nuclei and endoplasm form the characteristic osteoblasts a little earlier and the ground substance deposited in the exoplasm does not stain with hæmatoxylin. In the development of white fibrous tissue the nucleus and endoplasm lie upon the bundles of anastomosing exoplasm and in the course of time the anastomoses break and the exoplasm splits to give rise to the individual fibrils of white fibrous tissue.

The study of the development of elastic tissue is less satisfactory, usually, however, it develops in the middle of the exoplasm, the fibrils being extremely delicate at first, and anastomose from the beginning. Elastic tissue never develops by itself, but always in conjunction with some collagenous tissue, embryonic or mature.

Development of the Connective-Tissue Syncytium in the Tadpole.

Through the kindness of Professor Harrison I am enabled to follow the formation of the connective-tissue syncytium from the mesenchyme in a set of perfect serial sections of the tadpole. The sections had all been stained in hæmatoxylin and congo red and were cut $7\frac{1}{2}$ μ thick.

In a tadpole 3 millimeters long the mesenchyme is well defined around the spinal cord and brain, on the ventral side of the head and around the chorda dorsalis. The individual cells are made up of large irregular clumps of protoplasm filled with yolk discs and pigment granules, which almost hide the nucleus. At times the cells are arranged in rows and two cells in apposition often appear to be joined. This, however, is not frequent, and when they are united in this way they form only a syncytial rod, for they are never united with cells on all sides to

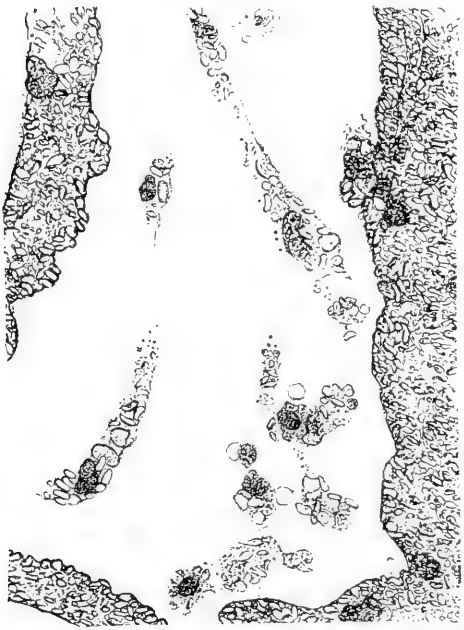

Fig. 1.

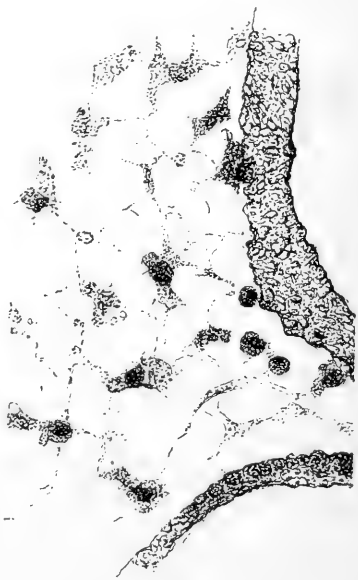

Fig. 2.

Fig. 1. Mesenchyme around the anterior end of the chorda of a tadpole 3 mm. long. Zeiss ob. 2 oc. 4 (× 500 diameters). Hæmatoxylin and congo red.

Fig. 2. Mesenchyme around the anterior end of the chorda of a tadpole 4 mm. long (× 500 diameters). The mesenchyme forms an extensive syncytium.

form a syncytial spherule. The best place to study the mesenchyme in this stage as well as in its further development is around the anterior end of the chorda, for here it is quite transparent and a distinct group of cells can be easily followed from stage to stage. Here the cells are large and irregular, as shown in Fig. 1. When these cells are examined carefully with a 2 mm. oil immersion lens it is seen that the nucleus is almost obscured by yolk discs and pigment granules. The

cell body itself is irregular in shape, running out into small elevations, or points, from which fine threads of protoplasm without pigment radiate. Occasionally one of these radiations reaches to and blends with a protoplasmic process from a neighboring cell. There is every indication of the beginning of an extensive syncytium formed by cells of the mesenchyme.

That the cells of the mesenchyme unite is a well known fact and can easily be demonstrated in frozen sections, and in teased specimens of the umbilical cord. In addition I need only to refer to the description and illustrations of Flemming[6] and of Spuler. "Die mit einander vielfach in netzartigem Zuzammenhang stehenden Zellen des Nabelstranges sind ueberwiegend spindelförmig odor 3-4 zipfelig und sind an den Enden in feinsten Fibrillen aufgefasert, bald dicht an der Zelle, bald erst nachdem ein Fortsatz sich ueber eine längere Strecke hin kompact erstreckt hat."[7]

The syncytium as seen in tadpoles 3 mm. long progresses rapidly to form a definite tissue from which only connective tissues arise. The mesenchyme has already divided into at least two groups of tissues in the embryo, one destined to form muscle and the other connective tissue. The syncytium destined to form the connective tissue, which I shall term the connective-tissue syncytium, begins to have its characteristic form at this time, and in its further growth it either remains as it is or gives rise to the connective tissues as we ordinarily understand them.

The point I wish to leave open is whether or not the mesenchyme was ever composed of individual cells. Was it not a syncytium throughout its development? The most valuable and suggestive studies of His[8] will have to answer this question for the present. At any rate, it is quite evident that the earlier syncytium, if it exists, is a very incomplete one, with very loose protoplasma bridges, easily broken and easily united to allow the cells to wander in all directions during the earliest stages of development. So it may be that the syncytium as seen in the tadpole 3 mm. long has existed ever since the appearance of the mesenchyme.

In a tadpole 4 mm. long the amount of mesenchyme, or connective-tissue syncytium, has increased a great deal around the brain, myotomes, etc. Around the anterior end of the chorda it is again very definite and can be studied better than elsewhere on account of its

[6] Flemming, His' Archiv, 1897, S. 183.

[7] Spuler, Anat. Hefte, viii, 133.

[8] His, Zellen und Syncytialbildung; Protoplasmastudien; Lecithoblast und Angioblast. Abhandl. d. K. S. Gesellschaft d. Wiss., Bd. 24, 25 u. 26, 1898–1900.

transparency. The main body of the cell mass has become decidedly multipolar in character and if anything, smaller than that in the embryo 3 mm. long. The yolk discs have largely disappeared while those remaining have become more transparent. On account of this change the nuclei are more distinct than in the earlier stage. The main cell body still contains many pigment granules. From each of the many poles of the cell fine threads of protoplasm arise, which divide once or twice, and anastomose into the same kind of threads from neighboring cells. In other words the multipolar cells form a complete syncytium. What now forms the main cell body gradually becomes a nodal point in an older stage of the syncytium. In this embryo we have mesenchyme pure and simple in the tail and a complete syncytium formed by the mesenchyme around the anterior end of the chorda. Between these there exist of course all intermediate stages.

In an embryo 6 mm. long no very great change has taken place in the development of the syncytium (Fig. 3). The cells in the tissue around the anterior end of the chorda appear much as in the earlier stage, with the exception that the protoplasmic bridges between the cell bodies are somewhat thicker and have a slight fibrillar structure, forming the first exoplasm. There are also some vacuoles in each process which indicate that an individual bridge is widening and breaking up into a number of bundles. The yolk discs and pigment granules are about as numerous and as definite as in the embryo 4 mm. long. The mesenchyme of the tail is now in the form of a complete syncytium on both its dorsal and ventral sides.

In another embryo slightly larger than the one just described and just before the mouth breaks through, the connective-tissue syncytium is of different arrangement in different portions of the body. Around the anterior end of the chorda the protoplasmic filaments of exoplasm form a dense network of fibrils a little more advanced than in the embryo 6 mm. long. They are now arranged as bundles between which there are numerous spaces. The endoplasm around the nucleus, including its transparent yolk discs and pigment, is spreading over the fibrillar network of exoplasm. It appears as if the main mass of endoplasm around the nucleus is being drawn upon to form more of the fibrillar exoplasm of the syncytium in its further growth. The nuclei can now be plainly seen lying upon or within the dense masses of fibrillar exoplasm of the syncytium.

In front of the brain the cells of the mesenchyme are spindle-shaped and run out into fibrils of thicker bands of protoplasm which form a

coarse network. In the mandibular arch all stages of embryonal connective tissue are seen, from single cells of mesenchyme, closely crowded together immediately below the ectoderm, to a complete syncytium lying deeper in the tissue. The single cells which are closely crowded undoubtedly form a growing point from which the syncytium arises. In the tail there is a very dense connective-tissue syncytium, more so than around the anterior end of the chorda. The nuclei are encircled with endoplasm which radiates over the exoplasm in all planes. Within the endoplasm there are imbedded numerous yolk discs and pigment granules; there are also some single yolk discs scattered throughout the

FIG. 3.

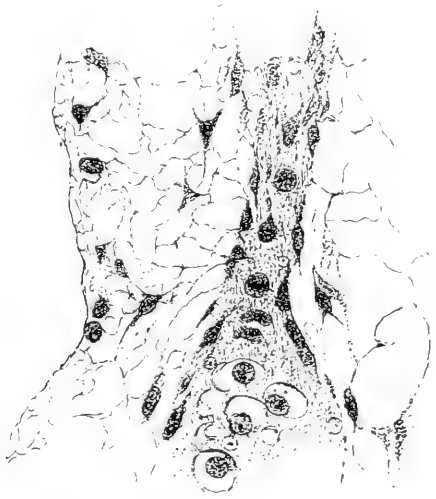

FIG. 4.

FIG. 3. Mesenchyme around the anterior end of the chorda in a tadpole 6 mm. long (× 500 diameters).

FIG. 4. The same in a tadpole 9 mm. long.

exoplasm, especially in the neighborhood of the yolk of the embryo. This condition occurs before the circulation of blood is well established, and indicates that the nutrition of the syncytium of the tail is carried on in part by the inwandering of cells from the yolk of the embryo.

In a stage somewhat older than the one just described, just after the mouth has broken through, the connective-tissue syncytium around the anterior end of the chorda is practically completed. Most of the nuclei, with a small amount of endoplasm around them, lie upon the exoplasm of the syncytium at its nodal points. Within the head in

front of the brain the exoplasm has increased markedly in quantity, by an addition to it from the mesenchyme cells at the growing point as well as by a multiplication of the cells of the finished syncytium. The same is true of the syncytium on the ventral side of the head. In the tail the connective-tissue syncytium forms a very dense network of exoplasm with nuclei and a small amount of endoplasm lying upon, or imbedded within, some of the nodal points. The endoplasm about the nuclei form stellate bodies with their points running out into the general mass of exoplasm. Minute pigment granules, often in rows, are distributed throughout the syncytium.

In general we have here a stage similar to that described by Flemming and by Spuler, and what I have stated above confirms the work of these investigators, though it formulates it somewhat differently.

The connective-tissue syncytium is practically complete in embryos 6 mm. long. In its further development it spreads and enlarges to form the general framework of the body. From now on there differentiate from it, with the exception of the chorda, the permanent connective tissues of the body, i. e., the skeleton, ligaments, tendons, true skin, etc.

Before discussing these tissues I shall describe the general arrangement of the syncytium in a tadpole 9 mm. long after the cartilages are beginning to form. In this embryo the syncytium around the anterior end of the chorda is again fully developed, with a difference, however, in the shape of the nuclei and endoplasm around them (Fig. 4). They are now spindle-shaped, lie upon and are connected with the exoplasm of the syncytium. In the course of time the nucleus and its endoplasm separates itself from the exoplasm of the syncytium, which is gradually converted into connective-tissue fibrils. The syncytium in front of the brain of the embryo is formed of bundles of anastomosing exoplasm with nuclei at some of the nodal points (Fig. 5). Each nucleus has a small quantity of endoplasm around it, forming a spindle-shaped mass which runs out into points to be lost in the exoplasm of the syncytium. In specimens of this kind it is easy to view these cells with their endoplasm as the connective-tissue cells and the exoplasm of the syncytium as the intercellular substance were not the development of the syncytium taken into consideration. Within the syncytium certain of the fibrils are more sharply defined than the rest, which indicates that in addition to the shifting of the nucleus and its envelope of endoplasm there is already some differentiation within the exoplasm. The syncytium in the ventral side of the head is much like that just described. As this is followed towards the tail there is a gradual transformation of

the arrangement of the bundles of exoplasm into an extremely dense network. In the tail the endoplasm around the nucleus forms a stellate mass with fibrils from the points running over into the fibrillar exoplasm of the main body of the syncytium (Fig. 6). Within the exoplasm there are some fibrils more sharply defined than the rest, which often appear to be composed of rows of extremely minute granules.

When the connective-tissue syncytium is fully developed in the tadpole it shows practically all of the characteristics found in mammalian embryos. I have made numerous chemical tests with the syncytium in the embryo pig, as an abundance of this material is constantly at my disposal. The tests were made with various stains, and digestive fer-

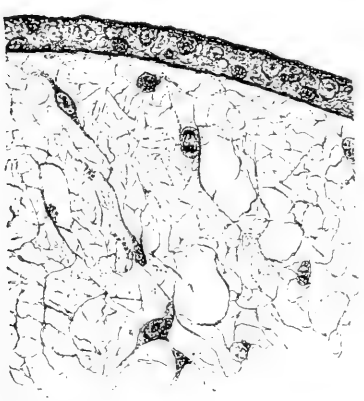

Fig. 5.

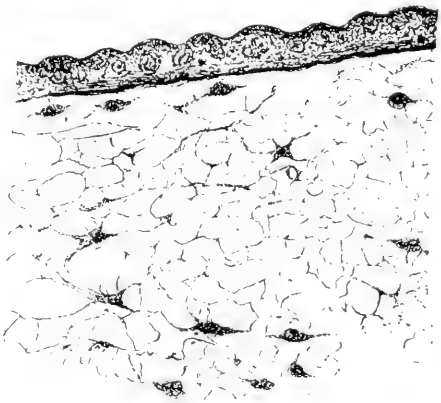

Fig. 6.

Fig. 5. Connective-tissue syncytium just below the ectoderm in the anterior part of the head of a tadpole 9 mm. long (× 500 diameters.)

Fig. 6. From the tail of the tadpole from which Fig. 5 was drawn.

ments upon sections which had been cut in paraffin. Frozen sections were also used a great deal, with more or less satisfactory results, to control the above, and to test with acetic acid, caustic potach, pancreatin, and pepsin.

The Connective-Tissue Syncytium in the Pig.

The connective-tissue syncytium is fully developed in the embryo pig from 9 to 12 mm. long. At this time it corresponds with that of the tadpole 6 mm. long. In the greater portion of the embryo, however, the syncytium is pretty well obscured by its numerous nuclei with the exception of that in the skin, around the brain and on the dorsal

side of the heart and lung. In these regions it is formed of an extensive network of exoplasm with nuclei at the nodal points. In other words, there are multipolar cells with anastomoses of their prolongations. At this time the nuclei are oval in shape, without the surrounding endoplasm as in the tadpole. At certain points there are indications of a beginning of a differentiation into cartilage and into white fibrous tissue, but beyond this there is the simple syncytium.

All the above may be seen in ordinary thin sections stained with acid fuchsin, but it is better shown in specimens stained with hæmatoxylin and congo red. My best specimens were obtained by staining the sections with Mallory's connective-tissue stain,[9] which tinges the nuclei and surrounding endoplasm, if present, slightly red and the exoplasm of the syncytium intensely blue. We have modified this stain somewhat by omitting the water and intensifying the blue. The method now employed in our laboratory, for which we are indebted to Dr. Sabin, is as follows:

Specimens hardened in Zenker's fluid are cut in paraffin and fixed on the glass slide by the water method. They are then stained with fuchsin, $\frac{1}{10}$ per cent, until they take up a proper amount of color and then without washing are fixed for a few minutes in a saturated aqueous solution of phosphomolybdic acid diluted ten times. They are next washed in alcohol, 95 per cent, and stained a very short time in the following solution: Aniline blue soluble in water, 1; orange G., 2; oxalic acid, 2; boiling water, 100. Next they are washed in alcohol, 95 per cent, blotted, cleared in xylol, and mounted in Canada balsam.

With this modification there seems to be no difficulty in obtaining an excellent double stain in nearly all cases, which is not so with the ordinary Mallory stain. Washing the sections with water has a tendency to remove the red and this is obviated to a great extent by substituting alcohol. The blue in this modification is strengthened in order that the section need not remain in the aqueous blue stain long enough to remove the red. Successful specimens are especially valuable to trace out the exoplasm of the syncytium which is somewhat matted together when stained with hæmatoxylin and congo red.

Digestion of the Syncytium in Pancreatin and Pepsin.

It is extremely difficult to obtain satisfactory results by digesting sections of embryos in either pancreatin or pepsin. If the test is made with frozen sections the pancreatin causes them to swell into a transpar-

[9] Mallory, Jour. of Exp. Med., Vol. 5, 1901.

ent slimy mass, difficult to handle or to stain in any very satisfactory way. In pepsin the section becomes firmer, opaque, brittle, and it must be crushed to separate the nuclei in order to study the syncytium, in case it is not digested. Quite satisfactory results are obtained by digesting sections of embryos, which have been attached to glass slides by the water method. It is of course necessary to use sections from embryos which have been hardened in alcohol, in order to obtain results similar to those obtained from frozen sections. Not only does this statement apply to embryos but to tissues in general. With pancreatin, however, the digestion upon the glass slide is very unsatisfactory, for the alkali in the solution nearly always detaches the sections, probably on account of the great amount of mucin in them. The younger the embryo the more difficult it is to retain the sections upon the glass slides.

Not only is it difficult to obtain fairly good sections which have been digested, but there is in addition the complication of unequal as well as contradictory results. When one point appeared to be worked out in a satisfactory manner, later tests contradicted it, and so on. It is therefore with some hesitancy that I give the tests with digestive ferments upon the connective-tissue syncytium.

In general it is quite certain that when the main mass of the syncytium is formed of exoplasm it is digestible in pancreatin and bicarbonate of soda. This treatment causes the section, if fresh, to become a swollen and slimy mass in which the delicate fibrils can be seen after it is treated with picric acid. The ground substance of the cartilage, if present, is well isolated and the more developed fibrils of the perichondrium can also be seen. It appears that the more the syncytium is differentiated the more it resists pancreatic digestion. In case a section of an older embryo is digested upon the glass slide it will be found that at the end of 24 hours all of the nuclei are dissolved, while all of the fibrils of the exoplasm of the syncytium, the white fibrils and the ground substance of the cartilage remain. In a section of this kind, which includes the umbilical cord, all stages of the syncytium can be studied; that in the cord is not differentiated, while that toward the back is changed into white fibrous tissue and cartilage. In the aorta there are at this time numerous elastic fibers. With Mallory's stain it is shown that a beautiful network of fibers alone remains, the nuclei having been digested in the pancreatin. Furthermore, it is shown by staining with Weigert's elastic tissue stain that the elastic fibrils have also been dissolved. The above-named tests were made many times on embryos from 7 mm. to 20 cm. in length. The older the tissue the

easier it is to isolate fibrils by digesting in pancreatin and bicarbonate of soda.

The action of pepsin and hydrochloric acid upon the connective-tissue syncytium appears to be just the opposite of that of pancreatin. The younger the syncytium the more difficult it is to digest it in pepsin. A section of a young embryo becomes opaque and shrinks a little when placed in dilute hydrochloric acid and pepsin. It is shown by examining it with the microscope at this time that the nuclei are opaque, and the white fibrous tissue, if present, has become transparent. After the sections have been kept in the digestive fluid for a few hours at 37° C. they still remain opaque and are somewhat elastic, for pressing a section under a coverglass only spreads it but does not break it. When stained with magenta the delicate fibrils of the syncytium are easily recognized. When the sections are digested for 24 hours or longer they usually fall into granules, showing that the syncytium is fully broken up. This is especially the case if the sections are from embryos 10 cm. long or longer. It is only in the smaller embryos that the syncytium is well developed and in them we are to make the valuable tests. The sections of an embryo 5 cm. long were still opaque at the end of 48 hours, with considerable elasticity, indicating that the syncytium must be present in considerable quantity. Furthermore, some fibrils could be seen from time to time in bits of crushed sections. The cartilage and white fibrous tissue of the perichordium resisted the action of the pepsin for 24 hours, but at the end of 48 hours they were fully dissolved.

Sections of embryos hardened in alcohol can be easily digested upon the glass slide and then stained with Mallory's connective-tissue stain or with Weigert's elastic-tissue stain. If the digestion is continued 24 or 48 hours usually all of the connective tissue and syncytium are dissolved, leaving only broken cells to outline the structures of the body. When the digestion is not complete it is found that usually the white fibrous tissue is dissolved first, then the cartilage and then the syncytium. A section through the body of an embryo, including the umbilical cord, has in it from the ventral to the dorsal side all stages of the syncytium in the process of differentiation. When such sections are digested to a proper degree, usually from 24 to 36 hours, it is often found that the white fibrous tissue of the body wall and back are dissolved while the syncytium of the cord is almost entirely intact; the cartilages are destroyed only in part.

It appears then that the connective-tissue syncytium is more resistant towards the action of pepsin than is white fibrous tissue. The more mucoid, that is, the younger, the syncytium is the more resistant it is

toward the action of pepsin. The action of pancreatin is in a measure the opposite.

Cartilage.

Cartilage appears as a band of condensed tissue on either side of the head of tadpoles just before the mouth breaks through. In the region destined to become cartilage the nuclei of the connective-tissue syncytium become first slightly enlarged, for nuclear figures are here more numerous. The endoplasm around the nuclei extends rapidly, and due to the multiplication of nuclei now fills the entire space and partly obscures the exoplasm of the syncytium. Where the endoplasm passes over into the exoplasm there is now a sharp line of demarcation making it appear as if the capsule of the cartilage cell were forming.

In tadpoles about 6 mm. long, immediately after the mouth has broken through, the nuclei of the precartilage are surrounded by a solid mass of endoplasm, thus filling up all the space between them. Where the nuclei are more separated the exoplasm is at the periphery of the endoplasm, but where the nuclei are packed together the exoplasm is wholly obscured. The endoplasm stains quite intensely with congo red, not more so, however, than the protoplasm of other cells.

A tadpole 9 mm. long has in it slender bands of cartilage fully developed from which the ground substance is directly continued into the exoplasm of the surrounding syncytium (Fig. 7). The best pictures are found at the tips of growing cartilage which are being added to by a transformation of the neighboring syncytium. Where the border of the cartilage is sharply defined the transition into the surrounding tissue is not marked, for its boundary line is obscured by a layer of flat cells. In a suitable specimen it is seen in passing from the syncytium over into the cartilage that the nuclei gradually become more and more crowded and the bundles of exoplasm become smaller and smaller. The nuclei gradually come to lie in the meshes formed by this exoplasm, that is, they have been extruded from the syncytium. With this change of relations between the nuclei and the exoplasm there is an increase of the endoplasm which now fills the meshes, encroaches upon the exoplasm, and partly obscures it. At this time the endoplasm of the syncytium stains with congo red, but as the finished cartilage is approached, the nuclei and surrounding endoplasm are separated by delicate lines or fibrils of exoplasm, which stain with hæmatoxylin. These lines now widen, stain more intensely with hæmatoxylin, and form the ground substance of the cartilage. The endoplasm becomes clearer and clearer, separates from the ground substance, and finally encircles

the nucleus only, leaving a space between it and the ground substance. In other words, we have a thickening and transformation of the exoplasm of the syncytium to form the ground substance of the cartilage, while the nuclei and endoplasm of the syncytium become the cartilage cells. This statement is found to be true in following the development of cartilage from embryo to embryo as well as in the transformation of the connective-tissue syncytium when cartilage grows into it.

The very early change in the syncytium preceding the formation of cartilage is much more easily followed in tadpoles than in mammals, the pig, for instance. On the other hand, when the cartilage is once formed its further growth is better studied in pig's embryo.

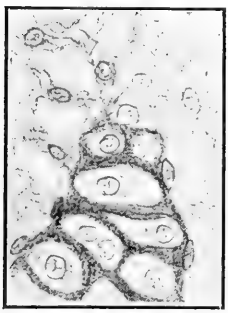

Fig. 7.

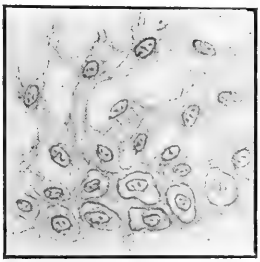

Fig. 8.

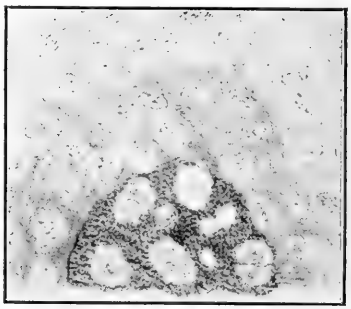

Fig. 9.

Fig. 7. Transition between cartilage and syncytium in a tadpole 9 mm. long. (× 250 diameters). Hæmatoxylin and eosin.

Fig. 8. Beginning of the occipital cartilage in a pig's embryo 16 mm. long (× 250 diameters). Mallory's stain.

Fig. 9. Transition between the syncytium and cartilage in an embryo pig 24 mm. long. The specimen had been macerated in Müller's fluid 24 hours before it was hardened in alcohol (× 250 diameters). Hæmatoxylin and congo red. The central dark zone stained with hæmatoxylin while the zone of ground substance between it and the syncytium only took the congo red.

The beginning of the formation of cartilage can be recognized in pig's embryos from 10 to 15 mm. long, in the condensed mass of cells around the chorda. When sections which have been stained in acid fuchsin are studied the connective-tissue syncytium can be followed to the areas of precartilage, but not into them, for the numerous nuclei obscure entirely the exoplasm of the syncytium. In sections stained by Mallory's method the beautiful and definite exoplasm of the syncytium can be followed to the precartilage, and between its numerous nuclei. As this tissue is followed from the region of the spinal cord towards the chorda it is found that exoplasm becomes denser and denser and the

nuclei somewhat larger with numerous karyokinetic figures. In the neighborhood of the chorda the exoplasm of the syncytium is so dense that it appears as a granular mass between the nuclei. The sheath of the chorda is stained intensely blue. The endoplasm which is quite marked around the nuclei of the syncytium near the spinal cord gradually disappears as the chorda is approached. By most methods of hardening the nuclei of the precartilage become so packed that the exoplasm of the syncytium is entirely obscured. Especially is this true in the development of the cartilage of the arm. In an embryo pig, 12 mm. long, which had been macerated in Müller's fluid for 24 hours, washed in water, then in alcohol, stained in hæmatoxylin and congo red, the precartilage of the arm could also be analyzed. The nuclei of the precartilage are all surrounded with a continuous mass of protoplasm, stained red, which is directly continuous with the exoplasm of the neighboring connective-tissue syncytium. In specimens which have been macerated it is very difficult to separate the endoplasm from the exoplasm of the syncytium. Therefore between the nuclei there is one continuous mass of protoplasm practically of the same structure.

In pigs' embryos from 15 to 20 mm. long the cartilages of the vertebral bodies are well developed and are pretty sharply defined. In sections treated with Mallory's stain it is found that on the dorsal side of the bodies of the vertebræ the ground substance of the cartilage is directly continuous with the exoplasm of the connective-tissue syncytium. By all odds the best place to study the early development of the cartilage is in the occipital cartilages, which lie on either side of the dorsal middle line. At this time the exoplasm of the connective-tissue syncytium in the region of the occipital precartilage is in the form of sharpened bands encircling definite openings, some of which contain nuclei (Fig. 8).

In a pig's embryo a little over 2 cm. long the main cartilages of the body are all well developed, and in this specimen we obtain the best pictures showing the relation of cartilage to the connective-tissue syncytium. Again, the occipital cartilage shows all the transitional stages from the completed ground substance to the exoplasm of the syncytium (Fig. 10). Passing from the ectoderm of the embryo towards the cartilage it is seen that the main spaces between the exoplasm of the syncytium become gradually smaller and smaller, with the nuclei shifting into them as the cartilage is approached. This takes place before any true ground substance is deposited, as the figure shows. Next we reach a zone in which the exoplasm has ground substance deposited between

its fibrils. Finally, when the cartilage is complete, the fibrils of the exoplasm are entirely obscured by the ground substance.

Sections stained by Mallory's method show that the endoplasm is almost wanting around the nuclei of the syncytium immediately below the ectoderm. Practically none is seen until the nuclei shift into the spaces between the exoplasm in the neighborhood of the cartilage. In this region the nuclei are larger than those more distant and in the region of the completed cartilage both nuclei and endoplasm are several times as large as in the surrounding syncytium. When the cartilage is fully developed the relatively large granular nuclei are encircled with vacuolated endoplasm. Each nucleus and endoplasm is encircled by a transparent space separating it from the surrounding exoplasm or ground substance. In specimens which have been macerated in Müller's fluid for a day, then washed and hardened in alcohol, and stained with hæmatoxylin and congo red, it is seen that no space exists between the endoplasm about the nucleus and the ground substance. By this method the endoplasm becomes more marked and the ground substance less marked than in the specimens hardened in Zenker's fluid and stained by Mallory's method.

Specimens of developing cartilage macerated as described above show at the juncture of the cartilage with the connective-tissue syncytium a zone of ground substance which will not stain with hæmatoxylin, but tinges with congo red (Fig. 9). Passing from the surrounding syncytium into the developing cartilage, the nuclei become larger, the exoplasm increases, condenses and obscures the endoplasm. Gradually the fibrillated exoplasm becomes granular, making it appear as if the nuclei were imbedded in a continuous granular mass. This all seems to be due to the maceration in Müller's fluid. On the periphery of the cartilage there is a zone of ground substance which does not stain with hæmatoxylin but tinges with congo red (Fig. 9). This zone is of the width of one or two nuclei which are surrounded with some endoplasm. The completed ground substance, which stains with hæmatoxylin, begins quite abruptly; the nuclei are encircled with a considerable quantity of endoplasm, filling almost entirely the spaces in which they lie.

The definite conclusion to be drawn from the above specimens is that the ground substance of the cartilage is deposited directly into the exoplasm of the syncytium and its nuclei and endoplasm become the cartilage cells.

Not only can the direct connection between the ground substance and the exoplasm be seen in the occipital cartilage, but also at the dorsal side of the bodies of the vertebræ, the petrous portion of the temporal

cartilage, and occasionally in other cartilages of the head as well as in the ribs. Otherwise the boundary line between the cartilage and the surrounding connective-tissue syncytium is quite sharp and is obliterated by the dense tissue and nuclei of the perichondrium. Specimens stained by Mallory's method are the best by all odds for studying the transition of syncytium into cartilage, for in them the ground substance and exoplasm are stained intensely blue, while the nuclei and endoplasm are shrunken and tinged red.

After the cartilage is once well formed its further growth is interstitial as well as peripheral. Not only do the nuclei divide, but the ground substance increases out of proportion, forcing the nuclei apart. This is beautifully illustrated in the sheath of the chorda, which is gradually incorporated with the vertebral cartilage. Often the thickened sheath appears as a great stump with its roots extending out into the cartilage.

In an embryo 3 cm. long the occipital and petrous cartilages have extended greatly, but still show beautifully the connection of the ground substance with the exoplasm of the syncytium. The transitions from the nuclei, endoplasm and exoplasm, into cartilage are again as distinct as in embryos 2 cm. long. This indicates that the syncytium at the periphery is being changed into and added to the cartilage already formed.

In older embryos the cartilage becomes separated more and more from the surrounding syncytium, with the exception where the cartilage is still extending into it. In an embryo 5 cm. long this condition is still present in the occipital cartilage and in the sternum. The borders of the vertebræ and most of the other cartilages are well defined and are beginning to ossify at different points.

Bone.

The frontal and mandibular are the first membranous bones to appear in embryos about 2 cm. long. In smaller embryos no indication of the formation of bone can be seen. Sections through the frontal region of embryos 2 cm. long stained by Mallory's method show that the bone begins by a very blue zone of hyaline deposit in the exoplasm of the connective-tissue syncytium (Fig. 11). The deposit appears to be equally distributed throughout the exoplasm within this zone. The nuclei stain somewhat more intensely than those of the surrounding syncytium, and the endoplasm around them is increased in quantity. Nuclei and endoplasm now show all the characteristics of osteoblasts and may be considered such. Sections certainly show definitely that when the syncytium turns into bone the nuclei become more sharply defined, the

endoplasm increases greatly in quantity, and the bone substance is either transformed exoplasm or is deposited into it. This latter process is first marked by the fibrils of the exoplasm becoming sharper and staining more intensely blue than before. Soon, however, the substance between the fibrils of the exoplasm takes up the blue stain, making it appear as if the tissue were injected with a blue color. In fact I thought for a long time that in this region there was an extravasation which stained blue, until I recognized the osteoblasts in older stages. Furthermore, the extravasation proved to be constant and took on the characteristic bone stain when treated with hæmatoxylin and congo red. The gradations in color from hæmatoxylin to congo red are in the order, nuclei, endoplasm, exoplasm, and bone substance.

The frontal and mandibular bones have increased in size in embryos 3 cm. long, the bone deposit is beginning to radiate into the surrounding

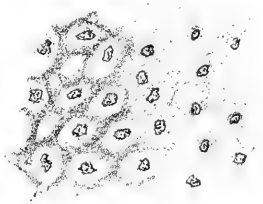

Fig. 10.

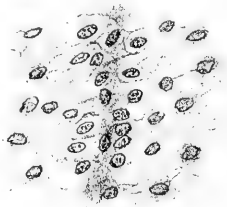

Fig. 11.

Fig. 10. Section through the occipital cartilage of an embryo pig 20 mm. long (× 250 diameters). The ground substance is deposited in the exoplasm of the syncytium.

Fig. 11. Section through the frontal bone of a pig 20 mm. long (× 250 diameters).

exoplasm, and the osteoblasts are larger and more numerous along these radiations. The bone radiations are still more pronounced in embryos 5 cm. long and are lost in the prefibrous tissue which is now arising from the surrounding exoplasm.

Most instructive specimens showing the extension of membranous bone as well as of the beginning of periosteal ossification are obtained from embryos 5 cm. long, stained by Mallory's method. In such specimens it is seen that the frontal bone is well started, with bone corpuscles imbedded within it, and osteoblasts encircling it. The bone substance with its radiations stains intensely blue, and it is seen that the radiations, with the accompanying osteoblasts, extend into the membranous skull and are gradually lost in the exoplasm and prefibrous tissue. At the extreme tips of the bone radiations the scattering fibrils are surrounded with osteoblasts. At the outer border of the

zone of osteoblasts, where there are transition forms between them and the nuclei of the syncytium, there are heavier fibrils of bone radiations between the nuclei. Passing from these fibrils towards the finished bone it is seen that they soon unite to form bundles which are soon stuck together to form the apparently homogeneous bone substance. After the bone is once well formed the primitive Haversian canals are filled with osteoblasts and a core of connective-tissue syncytium in which course the blood-vessels.

The difference in appearance between the beginning of bone in embryos 2 and 5 cm. long is probably due to the perfect syncytium in the former and the syncytium differentiating into prefibrous tissue in the latter. In the first the bone is deposited directly in the exoplasm, while in the second the exoplasm is first partly changed into prefibrous tissue and then into bone.

There are marked changes in the cartilages of embryos 5 cm. long, preparatory to their ossification. The shafts of the clavicle and ribs are encircled with a shell of bone and the transverse processes of the vertebræ are beginning to ossify. In suitable sections of the latter it is seen that the perichondrium is thickened and filled with osteoblasts. Not only do the osteoblasts appear to be arising from the nuclei of the perichondrium, but also from the outer layer of cartilage cells. Between the osteoblasts the bone fibrils first appear; they stain intensely blue with Mallory's stain, more so than the ground substance of the cartilage, extend throughout the perichondrium and extend somewhat into the cartilage. In order to separate the bone fibrils from cartilage it is necessary to stain sections with hæmatoxylin and congo red. Such sections show that the greater part of the bone is first deposited in the perichondrium, and only a small amount, if any, in the ground substance of the periphery of the cartilage, in periosteal ossification.

So it appears that in periosteal ossification the connective-tissue syncytium changes partly into cartilage and partly into white fibrous tissue before it gives rise to bone. Possibly the study of some suitable sections from embryos smaller than 5 cm. will give results identical with those obtained for the frontal bone, but so far I have been unable to obtain such sections.

White Fibrous Tissue.

In the study of the development of cartilage and bone definite spots can be located and followed from stage to stage. To do the same with white fibrous tissue and the other connective-tissue fibrils is much more difficult. Finally, after trying many regions, I settled on the develop-

ment of the connective tissues in the skin on the dorsal side of the body, between the two shoulder blades. Here there is the underlying broad trapezius, which marks a region in the section of the skin on one side with the epidermis on the other. Some 50 stages of this region were cut from embryos measuring from 1 to 30 cm. All important stages were hardened in Zenker's fluid, stained by Mallory's method, as well as by other methods. A parallel set of specimens was also made by macerating them in Müller's fluid for 24 hours, washing, hardening, etc., then staining in hæmatoxylin and congo red. All the stages were also frozen, cut and examined fresh, then treated with dilute acetic acid and with caustic potash, after which they were stained with magenta and other aniline dyes. While the specimens stained by Mallory's method give the most definite and permanent preparations, the macerated and fresh preparations give an excellent control. Mallory's method stains pretty much all connective tissues in the embryos, while with macerations and digestions there is some differentiation.

It is shown by means of digestion in pancreatin that the earliest definite fibrils in the syncytial exoplasm are resistant in fresh specimens as well as in hardened specimens which have been fixed upon the glass slide. In acetic acid and in caustic potash the exoplasm and its collagenous derivatives become transparent in embryos 25 mm. long. In larger embryos there is a residual syncytium which resists acetic acid, even when boiled in it for hours, and probably is related in some way to yellow elastic tissue. This will be considered later.

In general the connective-tissue syncytium is fully developed in embryos 15 mm. long. It is practically of equal density throughout the skin. The nuclei are mostly round or somewhat oval, usually quite naked or with only a small amount of endoplasm around them. The exoplasm is very delicate, with a slight amount of fibrillation.

In an embryo 2 cm. long the connective-tissue syncytium has begun to differentiate; many nuclei are oval in shape and they are enveloped with an increased quantity of endoplasm, which runs out on either side of the nucleus, forming two poles—the well-known connective "tissue cells" of the embryo. The nuclei lying just below the ectoderm have the least quantity of endoplasm around them. As the muscle lying under the skin is approached the nuclei increase in number, forming quite a dense layer over it. Analyzing this layer by means of Mallory's stain shows that it is formed of syncytium which is drawn out parallel with the long axis of the body. The fibrillated exoplasm tends to form parallel bundles which anastomose quite frequently with one another. In this region the nuclei are markedly spindle-shaped, with the

surrounding endoplasm running out into two poles to be lost in the exoplasm.

Digesting the syncytium and the prefibrous tissue in pancreatin shows that they resist its action to a marked extent. In order to obtain any satisfactory result the digestion must be mild, i. e., for a short time at room temperature. Great quantities of resistant white fibers cannot be obtained from the skin by means of digestion in pancreatin until the embryo is about 15 cm. long. Although no elastic fibers can be demonstrated in the skin of embryos 15 cm. long, frozen sections of it will resist boiling acetic acid for a very long time. In embryos 25 mm. long the first prefibrous tissue of the perimysium resists pancreatin more than the remaining syncytium. The fibrils of the prefibrous tissue also swell in dilute actic acid. These reactions, together with the position of the tissue in question, make it very definite that the changes in the syncytium immediately over the muscle mark the beginning of white fibrous tissue.

The prefibrous tissue of an embryo 2 cm. long is formed of anastomosing fibrils which are in direct connection with the exoplasm lying between the perimysium and the ectoderm. Immediately below the ectoderm the meshes of the exoplasm are smaller, the syncytium thus forming a more compact felt upon which the epithelium rests.

In embryos 3 cm. long the perimysium is a little more compact and sends reflections between the muscle fasciculi. The layer immediately below the ectoderm is a little more extensive than before, while between it and the perimysium the syncytium is quite typical. The two zones of altered exoplasm have approached each other in an embryo 4 cm. long, leaving a narrow zone of typical syncytium between them, within which the first lymph channels have appeared.

In the next stage, 5 cm. long, all of the exoplasm of the syncytium of the skin has changed considerably, being more fibrillated, with some of the fibrils staining more intensely than the rest (Fig. 12). The prefibrous perimysium is advanced one step more now, being composed of layers of fibrillated exoplasm, the layers anastomosing between themselves, and the fibrils within a given layer forming a dense network. True white fibrous tissue is not yet present.

The prefibrous tissue has extended still more in an embryo 7 cm. long. Its development is most advanced in the perimysium, where the individual fibrils are beginning to become wavy. In a transverse section of the body it is seen that the development is most advanced in the neighborhood of the vertebral column and least in the umbilical cord. From without inward the permysium is most developed, diminishing in

the intermuscular septa, ligaments, superficial fascia, and cutis, the least development being immediately below the epidermis. Even here it is no longer typical syncytium, but partly differentiated. The process continues in embryos 9, 10 and 12 cm. long; most of the prefibrous tissue is still within the syncytium. Immediately over the muscle a further differentiation of the prefibrous tissue of the perimysium has taken place. In this narrow zone the fibrils are arranged in parallel bundles apparently communicating with one another as well as being continuous with the neighboring exoplasm. In this region the individual fibrils anastomose with one another. Prefibrous tissue is changing into fibrous tissue very rapidly in an embryo 16 cm. long. The perimysium, composed of parallel fibers, sends processes of wavy fibers into the superficial fascia, and from them fibers enter the cutis. All of the tissue between the epidermis and the underlying muscle is composed of these

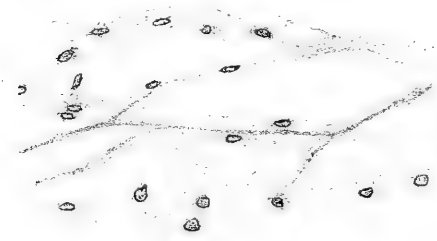

Fig. 12.

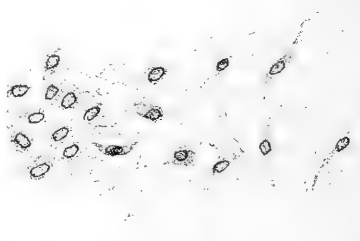

Fig. 13.

Fig. 12. Section through the skin of a pig 5 cm. long (× 250 diameters). The first white fibers are just forming in the exoplasm.

Fig. 13. Section through the skin of a pig 16 cm. long (× 250 diameters). The nuclei and endoplasm on the left are immediately below the root of a hair.

wavy fibers, either isolated or connected with the exoplasm, which is very fibrillated. The process is quite complete in embryos from 20 to 30 cm. long. In the older embryos, however, the density of the fibers is greater in the skin than in the underlying superficial fascia.

The individual fibrils after once well formed are of unequal size, often appearing in bands and frequently anastomosing. The anastomoses are finally broken and the bands and thicker fibers split into the individual fibrils.

I have now followed the development of the white fibers from the exoplasm of the syncytium without considering the nuclei and the endoplasm. What follows relates to them.

In smaller embryos (2 cm.), which have been stained by Mallory's method, the nuclei are round or oval with a small amount of endoplasm around them. When the tissue is macerated in Müller's fluid for 24

hours before cutting, the amount of endoplasm around the nucleus is greatly increased, showing that by Mallory's method it becomes shrunken. As the embryo grows the endoplasm increases in quantity until in specimens 5 cm. long it forms a spindle-shaped mass around the nucleus, the tips of which run out into the exoplasm and are lost. Hand in hand with the expansion of the exoplasm the endoplasm continues to grow until in embryos 10 to 12 cm. long it is differentiated to correspond with that of the exoplasm. In the neighborhood of the first white fibers the nuclei and endoplasm are arranged in rows, while, where the exoplasm is changing into prefibrous tissue, they are as before. More towards the epidermis the spindle-shaped endoplasm is much larger, indicating that it is also active in the conversion of exoplasm into prefibrous tissue. At this time the hairs are beginning to develop and below their roots the nuclei are multiplying and accumulating, apparently preparing much new endoplasm and exoplasm at these points (Fig. 13). At any rate, in larger embryos (15-20 cm.), there are islands of new syncytium at the roots of the embryonic hairs, making it appear as if the soft syncytium is present at these points to enable the hairs, in their further growth, to sink into the skin with greater ease. In the rest of the skin the embryonic white fibers, the prefibrous tissue, and fibrillated exoplasm are accompanied with nuclei surrounded with a spindle-shaped mass of endoplasm. Not only are all of these stages seen in the skin, but also between the radiations of embryonic white fibers from the perimysium into the superficial fascia.

After the activity of the nuclei and endoplasm has produced enough exoplasm to give rise to all the white fibers of the skin, which is the case in embryos from 20 to 30 cm. long, they cease to be so prominent and sink back into the form of irregular cells. Around the roots of the hairs there are still the islands of quite typical syncytium. Probably in both the scattered cells (nuclei and endoplasm) as well as in the islands of syncytium we have forces which can develop new white fibers, should circumstances so demand.[10] The syncytium at the roots of the hairs undergoes a further differentiation in the development of elastic tissue, which I shall take up presently.

It appears then that the connective-tissue syncytium grows rapidly before it gives rise to white fibrous tissue. The nuclei multiply, the

[10] Reddinghaus (Ziegler's Beiträge, 29, 1901) has shown that in inflammation of the omentum the fixed cells become active and form a syncytium which is in every respect identical with the connective tissue syncytium of the embryo. His pictures are in every respect like the normal specimens I obtained with Mallory's method.

endoplasm becomes larger, the exoplasm increases absolutely and relatively in quantity. The nuclei and endoplasm form the well-known bipolar cells, the tips of which run into and are lost in the exoplasm, making it appear as if the exoplasm were spinning its fibrils from the granular endoplasm. Soon the fibrillated exoplasm is drawn out into bundles, the bands between them beginning to break, thus forming the prefibrous tissue. The process of drawing out continues and the prefibrous tissue is changed into embryonic white fibers, which at first are irregular in size and anastomose occasionally. In the further development the bridges break and the thicker fibers split into the individual fibrils of white fibrous tissue.

The first white fibers appear in the perimysium, then they grow as radiations into the superficial fascia and cutis. Not only do the nuclei of the syncytium multiply, but the exoplasm increases much out of proportion. This continues in the prefibrous and embryonic fibrous tissues by stretching, widening, and splitting the individual fibrils.

Reticulum.

The reticulum of the lymph node is developed directly from the connective-tissue syncytium, and is probably the least differentiated of the connective tissues. This view has been advanced by writers, most recently by Waldeyer. In its differentiation it begins much like white fibrous tissue and when fully developed is about as far advanced as the tissue I have termed prefibrous. When white fibrous tissue and reticulum develop side by side it is impossible to separate them in their early stages, but when the early development of the perimysium is compared with the development of reticulum of a lymph node it is noticed that the arrangement of the fibrils is different, although their development is parallel. In the liver the reticulum develops from Kupffer's cells.

The development of the reticulum of the lymph node is now under investigation by Dr. Sabin who has given me the following résumé with the permission to publish it. "The lymph node has just appeared as a plexiform mass of lymph ducts in embryo pigs 4 cm. long. These ducts can be injected from more distant lymph channels and within the node they are relatively large and are separated from one another by bridges of tissue, or primitive lymph cords. The lymph cords are composed of a syncytium of delicate bands of exoplasm, with oval nuclei surrounded by spindle-shaped endoplasm. In addition there are many round cells which lie in the meshes—the first lymph cells. By the time the embryo is 10 cm. long the lymph node is one millimeter in diameter.

The whole node is composed of a delicate syncytium which now shows all of the characteristics of a fully developed reticulum, with many nuclei and endoplasm lying upon it. The meshes are partly filled with lymph cells. At the surface of the node the reticulum is continuous with the syncytium of the surrounding tissue. That there is a continuous network is best seen in sections stained by Mallory's method, which also show that the meshes are smaller and the fibrils are more delicate than those of the surrounding syncytium.

"The node has grown to be 3 mm. in diameter in embryos 20 cm. long. Each node is now surrounded with a delicate capsule of prefibrous tissue, and the reticulum, prefibrous tissue and surrounding syncytium form one continuous network. Upon the reticulum there are but few spindle cells and within the meshes there are many lymph cells."

From the above description it is seen that the reticulum develops directly from the exoplasm of the syncytium, while the nuclei and endoplasm are converted into cells which lie upon the reticulum fibrils. After the node is outlined the surrounding syncytium develops into prefibrous tissue to form the capsule.

The study of sections of the pig's intestine stained by Mallory's method shows definitely that both white fibrous tissue and reticulum are developed directly from the syncytium lying between the muscle wall and the epithelium. In embryos 20 cm. long there are small villi and rudimentary crypts present, but there is no marked muscularis mucosæ to separate the submucosa from the mucosa. There is no line of demarcation between the reticulum of the mucosa and the white fibrous tissue of the submucosa, more than a few scattered muscle cells of the muscularis mucosæ. The tissue around the bases of the embryonic crypts is fibrillated, wavy, and generally parallel with the muscularis mucosæ, stains more intensely and corresponds with the prefibrous tissue found elsewhere. From this layer there are gradual gradations towards reticulum in the villi on one side to a less developed white fibrous tissue in the submucosa on the other side. The degree of development of the layer of prefibrous tissue of the intestine is about the same as that of the skin of the same embryo.

The results here given suggest very much that reticulum represents an embryonic form of white fibrous tissue. That these two tissues blend and arise from a common syncytium does not speak for their identity any more than it does for the identity of either cartilage or bone with white fibrous tissue. As the matter now stands all of these tissues, including that of the cornea, are to be classed as collagenous, but still as distinct tissues. I have recently given the reasons for classing

reticulum as a separate tissue and will not enter upon the discussion[11] of this subject at present. At any rate, if these reasons are overcome, reticulum will remain as peculiar white fibrous tissue not fully developed, in case we can consider any tissue in the adult body as embryonic.

In examining various tissues for the development of reticulum, I found that in the liver it arises from Kupffer's endothelial cells, which here also form a beautiful syncytium.[12]

Frozen sections of the liver of a pig 2 cm. long are very delicate, and can easily be crushed under a coverglass. When such preparations are stained with a little magenta it is seen that a network of fibrils lies between clumps of liver cells. It can now be determined that all of the fibrils surround the capillaries and are formed by prolongations from Kupffer's cells. The fibrils, or rather the syncytium, is delicate, can easily be stretched and broken by slight pressure upon the cover glass. Such sections are also very easily broken into granules by giving them a delicate shake in water. When digested a short time in pancreatin at room temperature the liver cells break up and fall out, leaving the delicate syncytium to which are attached many small granules. In such preparations the syncytium is still very elastic and does not appear to swell in acetic acid.

The observations upon the development of the reticulum of the liver are entirely out of harmony with those of the development of connective tissue elsewhere. In all other places the syncytium arises from the mesenchyme but here it is from the endothelial lining of blood-vessels.

It is not difficult to obtain fresh specimens with all the capillaries surrounded with this syncytium which has the nuclei imbedded in it; the union is so complete that it is impossible to consider the nuclei and exoplasm in apposition only. The fibrils are in no way connected with the liver cells and true mesenchyme cells are not present at all.

The Cornea.

The cornea of a pig 2 cm. long is composed of a dense syncytium. The exoplasm is fibrillated and it radiates from nodal points where are located the nuclei and endoplasm. In an embryo 3 cm. long the general direction of the fibrils of the exoplasm is parallel with the surface of the cornea, i. e., the lamellæ are beginning to form. Between these primitive lamellæ the nuclei lie and are surrounded by

[11] Mall, Zeit. f. Morpholog. u. Anthropol., ii, 9.
[12] Kupffer, Archiv f. mik. Anat., 54.

spindle-shaped masses of endoplasm. A faint Descement's membrane is shown in specimens stained by Mallory's method; it does not stain by Weigert's method. Practically the same condition is found in the cornea of pigs 4 cm. long.

In an embryo 6 cm. long the cornea has grown in thickness, the quantity of exoplasm has increased and the nuclei have multiplied. The general character of the exoplasm is as before. In the cornea of pigs 9 cm. long the adult condition is present, the lamellæ of the anterior portion of the cornea being more developed than those of the posterior. The exoplasm forms definite lamellæ in the cornea of pigs 14 cm. long. The fibrillated lamellæ are bound together by bridges which run between them. Descement's membrane is sharply defined, stains intensely blue by Mallory's method, but does not stain by Weigert's method. It gives the same reactions in the cornea of the adult.

No elastic tissue can be demonstrated in the cornea of the adult either by Weigert's method or by treating frozen sections with boiling acetic acid and magenta. The lamellæ of the cornea can be easily resolved into fibrils by forming artificial œdema or by spreading frozen sections. In specimens made in this way the endoplasm is seen to encircle the nuclei and forms an extensive syncytium, as is well known. The tissue of the cornea contains much mucin and has often been spoken of as an embryonic connective tissue. It appears to be the only collagenous tissue which contains no accompanying elastic fibers. In many respects the cornea resembles the perimysium of the embryo before the white fibers have been fully formed from the exoplasm of the syncytium. At this time there are also no elastic fibers in the perimysium.

Elastic Tissue.

It is quite evident that in order to obtain any definite ideas regarding the development of elastic tissue it must be studied when it first appears. Studying its extension when once formed may give results which are misleading, for in older embryos the tissues which are being invaded have also undergone development.

In order to study the first appearance of elastic tissue I first tried to follow it in the skin, both human and pig's, for here I obtained the best preparations of developing white fibrous tissue. Furthermore, pieces of skin are easily cut by the freezing method and treated with the reagents usually employed in studying the connective-tissue fibrils. Although numerous tests and specimens were made the results were unsatisfactory until I had gained clearer pictures of the development

of elastic tissue in the arteries and in cartilage. For this reason I shall consider the development of elastic tissue in the skin at the end of the discussion.

Arteries.—At first it was extremely difficult to obtain any clear pictures of young elastic fibers in the walls of the arteries by means of Weigert's method, for the surrounding tissues were also stained somewhat black. Finally by staining the paraffin section upon the glass slide just long enough, complete differentiation was obtained by subsequent treatment with alcohol and hydrochloric acid, stronger than usual, and with a saturated aqueous solution of picric acid. By this method numerous sections were obtained with the elastic tissue only stained black. These were then counterstained with congo red or first with a very dilute solution of Delafield's hæmatoxylin to tinge the nuclei a little and then with congo red. In this way perfect specimens were obtained with the nuclei stained with hæmatoxylin, the elastic fibers stained intensely blue and the rest of the protoplasm red.

Not any elastic fibers could be demonstrated by Weigert's method in embryos less than 4 cm. long. As soon as the embryo has grown to this length a delicate network of elastic fibrils is stained intensely blue-black in the aorta and extends from the origin of the aorta into the arteries arising from it. Here they are gradually lost. The arteries of the skin do not have any elastic tissue in them. In a section of the carotid artery it is seen that there is a thick layer of elastic fibrils in the intima forming nearly a complete membrane. The media is but a few cells thick with a few individual fibrils between them. There are no elastic fibrils in the adventitia.

In an embryo 5 cm. long the elastic tissue is in the walls of the subdivisions of the main branches arising from the aorta. The walls of the whole aorta and its main branches are filled with fibers which extend into the adventitia. In the intima of the aorta the fibrils have coalesced to form the well-known fenestrated membrane. In the carotid the individual fibrils are present in the intima, the fenestrated membrane appearing in an embryo somewhat older. The muscularis is filled with most delicate elastic fibrils which together make a network of meshes which are filled with nuclei. At the outer border of the muscularis there is a gradual transition of the elastic tissue into the connective-tissue syncytium of the adventitia. In a thin section stained with Weigert's method and counterstained with congo red the relation of the elastic fibers to the syncytium is especially well seen when examined with the 2 mm. oil immersion lens of Zeiss. The elastic fibrils lie within the exoplasm together with other fibrils and the spindle-shaped

nuclei and endoplasm lie upon these bundles. The degree of development of the exoplasm is practically of the stage I have termed prefibrous above with numerous elastic fibrils, which stain with Weigert's stain, added. This process is slightly more advanced in the umbilical artery, which is especially suited for the study of early elastic fibrils, in longitudinal or oblique sections (Fig. 14). In such sections all grades of the development of elastic fibrils are easily found—from perfect syncytium in the cord without elastic fibers to the finished elastic tissue in the intima. At the point of juncture between the media and the adventitia it is seen that the white fibrous tissue gradually passes over

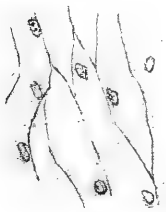

Fig. 14.

Fig. 15.

Fig. 14. Elastic tissue just beginning in the syncytium of the umbilical vein of a pig 7 cm. long (×250 diameters). The specimen was first stained by Weigert's method, then tinged with hæmatoxylin and counterstained with congo red.

Fig. 15. Elastic fibers isolated from the skin of a pig 16 cm. long by means of boiling acetic acid (×250 diameters). Stained with gentian violet. The fibrils form baskets around the bundles of white fibrous tissue which are converted into a jelly-like mass.

into prefibrous tissue and this in turn over into typical exoplasm of the syncytium of the cord. The degree of development of the elastic tissue is exactly parallel with this. In the media the elastic network encircles the bundles of white fibers, while in the region of prefibrous tissue the network is in the periphery of the exoplasm. Farther out, in the adventitia, the network of elastic fibrils is all through the exoplasm.

The fibrillated exoplasm in the walls of the arteries is composed of two kinds of fibrils, destined to become the fibrils of white fibrous and yellow elastic tissue. As this process of differentiation begins the white fibers swell in acetic acid, are not digested in pancreatin, etc. While the yellow elastic fibrils resist acids and dilute solutions of potassium hydrate and stain intensely when treated by Weigert's method. At

first the elastic fibrils form a network throughout the exoplasm but they gradually shift to its outer border, leaving the prefibrous tissue within. At this time the nuclei and endoplasm lie upon the exoplasm. A further development liberates the nuclei and endoplasm more and more and the elastic fibers come to form a network which encircles bundles of white fibers, to form the characteristic and fully developed connective tissue.

The youngest elastic fibrils which are stained by Weigert's method form a delicate network of homogeneous fibrils less than one μ thick (smaller than the chromatin granule of the nucleus), and at no time are the fibrils composed of a row of granules as described by Ranvier. Ranvier's description of elastic granules in arytenoid cartilage is correct so far as it goes but does not apply to the development of elastic fibers.

I have studied carefully the development of elastic tissue in an embryo 7 cm. long, which had been hardened in alcohol, thus permitting tests with various digestion ferments. This was not possible with most of the sections I studied, for they were from embryos hardened in Zenker's fluid. Thin sections of the aorta show, when stained by Mallory's method, a beautiful syncytium composed of fibrillated exoplasm within which there is a network of sharply defined fibrils which stain intensely blue. The elastic membrane of the intima is also stained intensely blue. The arrangement of the network which stains more intensely by Mallory's method is identical with that stained by Weigert's method. If, now, a section is first digested in pancreatin for 24 or 48 hours, the network of the syncytium and the membrane of the intima are no longer present, as is shown in sections which have been stained with either Mallory's or Weigert's method. A shining mass of anastomosing fibrils of the exoplasm alone remains intact, nuclei, endoplasm, and elastic fibrils having been removed by the action of the pancreatin. From time to time specimens may be obtained by the action of pepsin in which only some elastic fibers and fragments of nuclei are left. In general, it is as difficult to isolate elastic fibers by the action of pepsin in the tissues of the embryo as it is to isolate them in the adult.

In thin sections of the embryo 7 cm. long which have been stained successfully with Weigert's elastic tissue stain, Delafield's hæmatoxylin and congo red the relation of white fibrous and yellow elastic tissue to the syncytium is beautifully shown in the adventitia. The two kinds of fibrils alternate in bundles with nuclei and endoplasm lying upon them. The individual elastic fibrils may appear as rows of granules, but the granules never leave the field of the microscope while focusing,

i. e., the granules are optical sections of fibrils of elastic tissue closely packed around the bundles of white fibers. In the adventitia of the umbilical artery, where the fibrils are cut parallel in this specimen, the fibrils are all homogeneous and continuous.

As the embryo grows the elastic tissue gradually extends along the arteries to every part of the body, reaching those of the skin in embryos about 20 cm. long. Shortly after the arteries of the skin have elastic tissue in their wall, it can also be demonstrated in the loose tissue below the hair follicles.

From the study of the development of elastic tissue in the arteries it is seen that the exoplasm of the connective-tissue syncytium forming their walls differentiates into two kinds of fibrils, which give rise to the white fibrous and elastic tissues, respectively. In other words, one cell gives rise to both tissues.

Arytenoid Cartilage.—In the arteries the elastic and white fibrous tissues develop at the same time from the common exoplasm, as would be expected in a region where the elastic tissue develops so early. In cartilage, on the other hand, the exoplasm is converted completely into the ground substance before the elastic fibers develop. A condition which is parallel with that in cartilage is found in the skin, in bone and in reticulated tissue when accompanied by elastic fibers.

The arytenoid cartilage of the adult pig is partly hyaline and partly elastic. Where the two kinds of tissue come together the fibrils course in the ground substance between the cartilage cells. The hyaline cartilage near the elastic is infiltrated with granules which are sometimes in rows but more frequently in clumps around one or more cartilage cells. Generally the granules in the ground substance lie midway between the cells but where they begin to form masses they are usually around a single cartilage cell. According to Ranvier the granules form rows which coalesce to form elastic fibers. My own observations show that whenever fibers or granules are in the same neighborhood that they are separated and that one is never continuous with the other. We have here to do with a special kind of elastic tissue composed only of granules, as we have another form in the fenestrated membrane in the smaller arteries. Conclusive proof is obtained when the development of these structures is followed in the embryo pig.

The arytenoid cartilage of a pig 12 cm long is a few millimeters long and can easily be dissected out. It is then to be frozen and cut, stained by Weigert's method, and mounted as usual. Such sections show that most of the cartilage is hyaline, with some elastic fibers appearing at one end of the cartilage. The fibrils are extremely delicate and lie

within the ground substance midway between the cells. At no point is the diameter of the fibers as great as that of the granules in the arytenoid cartilage of the adult. Furthermore, there are absolutely no granules of elastic tissue in the cartilage in which the elastic fibers have appeared and are growing. The same pictures, only more advanced, are seen in the arytenoid cartilages of pigs' embryos up to 24 cm. long. I have been unable to obtain specimens between embryos 24 cm. long and the adult, so cannot contribute anything regarding the development of the elastic granules. It is probable that they appear as minute specks and gradually grow larger and larger, for where they are in clumps granules of all sizes are seen.

Mucous Membrane of the Intestine.—The reticulum of the mucosa and the prefibrous tissue of the submucosa form a single layer in the intestine of the embryo pig 24 cm. long. At this time no elastic fibers whatever can be demonstrated in any of the layers of the intestine by Weigert's method. Unfortunately the succeeding stages were not at my disposal, but from the examination of the intestine in the adult it is shown that the bundle of white fibers of the submucosa are surrounded with numerous elastic fibers which form a dense network throughout the muscularis mucosæ and the stratum fibrosum. From this point a few fibrils extend between the crypts but not into the villi. Spalteholz[13] has followed them throughout the mucosa, showing that they accompany the muscle bundles of the villi. At any rate there is considerable reticulum in the mucosa of the intestine which has no accompanying elastic fibers, as is also the case in the ground substance of cartilage.

Lymph Nodes.—Frozen sections of lymph nodes which have been stained by Weigert's method show beautiful networks of elastic fibers throughout the trabeculæ and the follicles. Within the trabeculæ the elastic fibrils are very numerous and from there they pass at regular intervals along the bands of reticulum through the sinus to the periphery of the follicle. Their course is quite direct towards the center of the follicle where they anastomose to form an irregular network. If the section is macerated for a few days in a solution of bicarbonate of soda to soften the cells, the sections can be cleared pretty well, leaving only the reticulum and the elastic fibers. When specimens thus obtained are stained by Weigert's method it is found that not all the reticulum fibrils are accompanied with elastic. At the periphery of the follicle about every second fibril, while more towards its center, about every fifth reticulum fibril is accompanied by an elastic fiber.

[13] Spalteholz, Arch. f. Anat., Supplement Band, 1897.

The examination of numerous thin sections cut in paraffin and stained by Weigert's method showed that the amount of elastic tissue in the follicle is by no means constant. Occasionally no fibrils at all could be demonstrated by this method while frequently they were only at the periphery of the follicle. Care must be taken in such tests not to stain the sections too long, for the reticulum, and the white fibrous tissue of the capsule, take up considerable stain and thus lead to confusion. The only definite tests are those in which the surrounding elastic tissue stains intensely, leaving the white fibrous tissue colorless. In a beautiful specimen of a Peyer's patch the elastic tissue accompanies every reticulum fibril into the follicle for two-thirds of the distance to its center and then ends quite abruptly. When not highly magnified it appears as if the reticulum itself were stained intensely, but with the 2 mm. oil immersion it is very apparent that each fibril of reticulum is encircled with several delicate elastic fibrils. At the center of the follicles there are no elastic fibrils at all. The variation in the amount of elastic tissue in the lymph node suggests at once whether it is not due to some pathological process, for most of my sections were from human lymph nodes which had been cut for other purposes. The recent work of Melnikow-Rasnednekow,[14] Flexner,[15] and others upon the formation of elastic tissue in cirrhosis of the liver suggests this view. The observations are sufficient, however, to show that elastic fibrils accompany some, but not all, of the reticulum fibrils in the follicle of the lymph node. Furthermore, the development of reticulum precedes that of elastic tissue.

Skin.—It is extremely difficult to obtain clear pictures of the development of elastic tissue of the skin, when the youngest fibers which take Weigert's stain are studied in relation to the nuclei or to the white fibers. Practically no better results are obtained from the embryo than from the adult. In each case there are sharply defined fibers and that is all. On the other hand, when the skin is macerated by boiling frozen sections in 1 per cent acetic acid until the white fibers are mostly dissolved or are converted into a jelly-like mass the relations are somewhat distorted but the results are instructive, when compared with sections of the skin and of the larger arteries which have been stained by Weigert's method.

The elastic tissue of the arteries of the skin stains by Weigert's method in embryos from 20-25 cm. long. There are no elastic fibers within the skin itself. The clear areas at the roots of the hairs are

[14] Melnikow-Rasnednekow, Ziegler's Beiträge, 26, 1899.

[15] Flexner, Univ. Med. Mag., 1900.

filled with nuclei encircled with endoplasm lying upon a delicate network of exoplasm. This is beautifully shown in specimens stained by Mallory's method, and also to a certain extent by Weigert's method, provided the stain is pushed until the surrounding white fibrous tissue stains also. In embryos a little over 25 cm. long the elastic tissue of the arteries of the skin has increased in quantity, and the exoplasm of the syncytium below the roots of the hairs undoubtedly is stained more readily by Weigert's method than before.

When frozen sections of the skin (which show no elastic tissue by Weigert's method) are boiled in dilute acetic acid until the white fibrous tissue is either dissolved or converted into a jelly mass, a network of sharp fibers can still be demonstrated by staining the swollen section with magenta or with very dilute gentian violet (Fig. 15). In case the sections are not boiled very long the gelatinous exoplasm of the syncytium has imbedded within it sharp fibrils upon which lie oval nuclei surrounded with a plate of endoplasm. When the boiling is pushed still further, until the section falls nearly into pieces, it can still be coaxed upon the glass slide and stained with magenta under the coverglass. The main bands of syncytium are now practically all dissolved, leaving a network of delicate and sharply defined fibrils which appear to be directly continuous with the endoplasm around the nuclei. Often some of the anastomosing fibrils are quite free and upon them lie the nuclei and surrounding endoplasm (Fig. 18). These specimens, which are extremely instructive, show definitely that the nuclei and endoplasm lie upon the fibers. Furthermore, when frozen sections are treated a short time in boiling dilute caustic potash only a network—the elastic fibers—remains, all the rest, including the nuclei, having been dissolved. These tests show that an elastic network is present in the skin in young embryos before it can be stained by Weigert's method. Elastic tissue can be demonstrated by Weigert's method in the skin of the embryo pig about 25 cm. long, and by maceration in boiling acetic acid, and staining with magenta, the fibrils can easily be isolated (Fig. 16). It is therefore seen that elastic tissue is present in the skin long before it can be stained by Weigert's method.

In a section of the skin in which the elastic fibers just begin to take the Weigert's stain it is seen that the bundles of white fibrous tissue are accompanied by one or two elastic fibers. In the region of the roots of the hairs, where the development is not so far advanced, the fibers are continued into the exoplasm of the syncytium and are related to the nuclei and endoplasm as described above. Frozen sections boiled in acetic acid (1 per cent) until very soft, then coaxed upon the glass

slide and stained with magenta, show that the elastic fibers are related to the exoplasm of the syncytium much as they are to the reticulum of the lymph follicle.

It is much more difficult to obtain specimens of the human skin in which the elastic tissue is just beginning to appear. Fresh specimens are not always at hand and preserved specimens are often unsuited to cut into frozen sections which can be boiled or macerated.

In the skin of a human fœtus, measuring 22 cm. from head to breech, practically no elastic fibers are stained by Weigert's method—much as in pig's embryos of the same length. Sections which have been boiled in dilute acetic acid for 4 hours had the white fibrous tissue de-

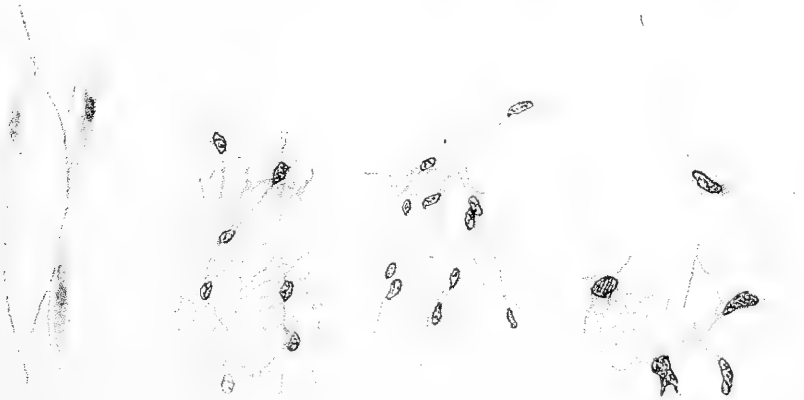

FIG. 16. FIG. 17. FIG. 18.

FIG. 16. Elastic fibers isolated from the skin of a pig 24 cm. long (×250 diameters). Magenta. The skin was frozen and cut, then boiled in acetic acid (1%) for one hour. The fibrils form baskets around swollen bundles of white fibers. To them cling nuclei and endoplasm.

FIG. 17. Elastic network obtained from the skin of a human foetus 22 cm. long (×250 diameters). Stained with magenta. The specimen had been hardened in alcohol, was washed in water, frozen, and cut. Sections were then boiled in acetic acid (1%) for 4 hours. Further treatment showed that the nuclei and endoplasm could be removed by means of dilute caustic potash, leaving only the delicate elastic fibers.

FIG. 18. Elastic fibers from the skin of a human fœtus 26 cm. long (×250 diameters). The fresh tissue was cut by the freezing method and boiled in acetic acid (1%) for an hour. It was then coaxed upon a slide and stained with magenta. All of the fibers have large nuclei clinging to them.

stroyed completely, leaving only a delicate network of fibers upon which the nuclei lie (Fig. 17). In this specimen it really seemed at first as if there is a complete network formed by the anastomoses of the ends of numerous multipolar cells, but crushing the section and pulling it apart, showed that a delicate network of fibrils remains, which stain with

magenta, is partly buried in the gelatinous remnant of the white fibrous tissue, and is partly covered with nuclei and endoplasm. The elastic network can be further isolated by boiling the section in a dilute solution of caustic potash; the delicate elastic fibers alone remain, the white fibers and nuclei having been removed completely.

The skin of a fœtus 7 months old (26 cm. long) has within it many delicate elastic fibers which are stained by Weigert's method. The individual fibrils are in general parallel with the bundles of white fibrils, are not composed of rows of individual granules, but are homogeneous. When the sections are boiled to remove the white fibers in part and then stained by Weigert's method, a beautiful network remains, one or two fibrils accompanying each swollen bundle of white fibrils. Frozen sections boiled in dilute acetic acid and stained with magenta give the same picture. The oval nuclei with the surrounding endoplasm lie upon the elastic fibrils, surround them, but are not continuous with them (Fig. 18). Similar results have been obtained by Jores, who studied the formation of elastic fibers in a myxoma.[16]

The elastic fibers have increased greatly in number in the skin of a fœtus 8 months old. The fibers are closely packed to form baskets encircling the individual bundles of white fibers. Specimens made by the aid of boiling acetic acid are again most instructive, for in such specimens the fibers are isolated with nuclei and endoplasm clinging to them. Thick sections made in this way appear as a felt in which there are numerous holes, where the bundles of white fibers lay, with nuclei and endoplasm clinging to the elastic fibers. In the skin at birth the elastic fibers have become a little larger and denser, and therefore more numerous as the skin has expanded and become thicker. Frozen sections which have been treated with boiling actic acid and stained in magenta show nuclei and endoplasm attached to the individual fibers. Sometimes they are spindle-shaped but usually they form plates which are easily separated from the elastic fibrils after the white fibers have been dissolved.

In the skin of an infant two months old the elastic and white fibrous tissues are about equal in quantity. The elastic fiber baskets encircle and frequently sink into the bundles of the white fibers, as is easily shown in sections which have been stained by Weigert's method. The same picture is seen in the skin of infants from two to six months old.

While the elastic fibers are present in relatively small number in the skin of a fœtus 22 cm. long, and gradually increase in size and

[16] Jores, Ziegler's Beiträge, xxvii, Fig. 3.

quantity as the fœtus grows older and after birth, the study of their development in this region gives unsatisfactory results. It is definite, however, that they always appear around the bundles of white fibers, being covered, especially at their points of anastomosis, with nuclei and endoplasm. If the early formation of elastic tissue in the syncytium of the walls of the umbilical artery is considered the type we must interpret what has been found in the skin as a secondary differentiation of the exoplasm, which is already collagenous, into elastic tissue, as is also the case in the ground substance of the cartilage. In the cartilage, however, the fibers develop in the middle of the ground substance, as far away from the nuclei as possible, while in the skin the elastic fibers appear at the periphery of the bundles of white fibers, close to the nuclei.[17] The same is true regarding the elastic fibers which are formed in the lymph follicle along some of the reticulum fibrils.

From this histogenetic study it must be concluded that elastic tissue is a more highly differentiated tissue accompanying to a greater or less extent all collagenous tissues (reticulum, cartilage, bone, and white fibrous tissue) with the exception of the cornea.

The study of the growth of all connective tissues is difficult, for after they are once differentiated and quite sharply separated from the nuclei and endoplasm they have then power of further growth and expansion without a continuous transformation of endoplasm into exoplasm.

[17] See also Jores, Ziegler's Beiträge, xxvii, Fig. 4.

ON THE ORIGIN OF THE LYMPHATIC SYSTEM FROM THE VEINS AND THE DEVELOPMENT OF THE LYMPH HEARTS AND THORACIC DUCT IN THE PIG.

BY

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WITH 12 TEXT FIGURES.

Although considerable attention has been given of late to the study of the development of lymph glands, only two writers have led up to the discovery of the origin of the lymphatic system as a whole, Budge[1] and Ranvier.[2]

In 1880 Budge published an account of a canal system which he had discovered in the mesoderm of early chick embryos; and in 1887, after Budge's death, His published a further but necessarily incomplete account of this work from Budge's notes and pictures.

Budge injected the false amnion of chicks three days old, and found that the fluid ran out into the area vasculosa as if in ducts. He then injected along the arteries in chicks from nine to eighteen days old and obtained beautiful injections of undoubted lymphatics. These two experiments are related to one another in the text by the following theory: Budge thought that there were two lymphatic systems, and that the first or primitive system was present in the three-day chick. He thought that the false amnion and cœlom being continuous, there were ducts within the body wall connected with the cœlom, analogous to those of the area vasculosa which he had injected from the false amnion. The ducts within the body lying along the dorsal line became pinched off from the cœlom and united to form a thoracic duct. With the thoracic duct began the second or permanent lymphatic system, which he had injected along the arteries in nine-day chicks. This idea of relating the lymphatic system to the serous cavities has remained but a theory and the gap between the two systems of Budge has never been filled.

[1] Budge: Arch. f. Anat. u. Phys., Anat. Abthg., 1880 and 1887.

[2] Ranvier: Comptes Rendus, 1895 and 1896; Archiv d'Anatomie, Tome 1, 1897.

Having repeated Budge's experiments I am convinced that while his injections of the ducts along the arteries are fundamental in the study of a certain stage of the development of lymphatics, the spaces connected with the false amnion have no connection whatever with the lymphatic system. In injecting the false amnion some minor changes in the methods of Budge are an advantage. Instead of a hypodermic syringe it is better to use a glass tube drawn out to a fine point and to introduce the fluid slowly under the even pressure of a low column of mercury. Budge used Berlin blue and found it necessary after filling the false amnion, to stroke the embryo gently in order to force the fluid into the area vasculosa. India ink is, however, a better fluid, for it is so finely divided that it runs of its own accord. I inserted the needle into the false amnion according to Budge's directions, given in his first paper, and injected the ink until the cavity was just full, then floated the embryo on to a glass slide with the dorsal surface upward. The fluid will now enter the area vasculosa and in places will run to its edge. This can be watched under the microscope. The fluid runs by putting out blunt processes simulating canals and so interpreted by Budge; at first these processes anastomose, making the network shown in Budge's pictures, but, as a rule, the meshes soon fill in and the fluid advances as a solid column with processes projecting in every direction. In other words, the fluid runs just as it would if forced between two glass plates held closely together. In serial sections through these injected specimens it is found that the upper and lower layers of the area vasculosa are connected here and there by delicate fibrils which are really processes of scattered mesenchyme cells and that the injected fluid passes into the spaces thus made and not into preformed channels lined by endothelium.

Notwithstanding the fact that Budge's theory of the origin of the thoracic duct is not correct, this theory led to the discovery of the true origin. For it was by injecting into the side of the neck in early embryos in the hope of reaching Budge's spaces behind the aorta, that the cervical lymph heart was injected, and the lymph hearts give the key to all the superficial lymphatics.

Between the years 1895 and 1897, Ranvier published a long series of articles on the development of the lymphatic system. He worked chiefly on the frog, and on pig embryos from 9 to 18 cm. long. From his injections of the lymphatic capillaries in the skin and in the villi of the pig embryos he made an important discovery; namely, that the lymphatics within the capillary plexus grow by budding. He says that

from the side of a duct appears a bud which is at first solid, but soon has a lumen. The lumen becomes larger, while at the same time the bud advances until it reaches a second duct. It opens into this duct by a process of absorption of the endothelium, and at the point of junction a valve is made. These points are beautifully seen in injected specimens for the larger duct forms, as Ranvier says, a collarette for the smaller. Thus he says the ducts grow from centre to periphery, while the valves necessarily open in the opposite direction. He says that as soon as lymphatics can be recognized in mammals they are furnished with valves. He also worked on frogs and described injections of the subcutaneous lymph sacs. From these sacs fluid can be made to run centrally to one of the lymph hearts and thence to the vein, and peripherally to minute ducts in the web of the feet. Ranvier states that these small ducts develop from the great lymph sacs.

His general conception of the lymphatic system is that it is a great gland of which the lymphatic capillaries correspond to the secreting portion, while the lymphatic ducts are the excretory canals. He says that the lymphatic system may be considered as a great vascular gland which takes its origin embryologically in the venous system and pours into the veins the product of its secretion which is lymph.[3]

Ranvier's great contribution to the study of the lymphatic system is the discovery of the fact of the growth of the ducts by budding in contradistinction to the generally accepted theory that the lymphatic system develops out of tissue spaces. Gulland[4] states this theory clearly as follows: The fluid of the blood exudes from the veins into the tissue spaces which gradually dilate, flow together and form the first lymphatic ducts. The walls of these ducts are made from the connective tissue which becomes compressed around them, and the ducts subsequently open into the veins. The most recent statement of this theory is that of Sala.[5] He describes the development of the lymph hearts and the thoracic duct in the chick as follows: That the first trace of the lymphatic system is the appearance of lymph hearts or spaces in the mesenchyme just lateral to the caudal myotomes. These spaces flow together and join the thoracic duct, which forms as two cords of mesenchyme cells which extend from the level of the thyroid glands to the level of the cœliac axis. In the centre of these

[3] Ranvier: Comptes Rendus, Tome 121, 1895, p. 1109.

[4] Gulland: Journal of Pathology and Bacteriology, Vol. II, 1894, p. 466.

[5] Sala: Ricerche n. lab. di anat. norm. d. r. Univ. di Roma, Vol. VII, 1900, pp. 263-269. Reviewed in Archives Italiennes de Biologie, Tome 34, 1900, p. 453.

cords develops the lumen of the thoracic ducts which forms connections with the ductus Botali, the aorta and the superior vena cava.

In the present communication, Ranvier's hypothesis that the lymphatic system takes its origin from the veins will be proved. He missed the proof because he thought there were no lymphatics in pig embryos under 9 cm.[6] As a matter of fact the ducts have spread over nearly the whole body in a pig 5.5 cm. long.

In the study herein reported, I have been greatly aided by a manuscript of Dr. W. G. MacCallum's which I was privileged to read. From this paper, which is on "The Relations between the Lymphatics and the Connective Tissue," and is soon to be published in the Archiv für Anatomie, certain observations and conclusions which aid my work are quoted with the author's permission.

He injected the subcutaneous lymphatics of embryo pigs for the most part between 5 and 15 cm. long and has given most graphic and accurate descriptions of these injections, and of the lymphatics both in fresh and stained preparations. He noted as Ranvier had the growth of the lymphatic capillaries within the plexus by budding, and describes the long sprouts or strands of endothelial cells growing out from the ducts, and how the lumen of a duct gradually opens into the sprouts. He discovered the fact that the early lymphatics have no valves, and made an important addition to the method of injection, by stripping off the skin, placing it on a slide and injecting it under the microscope. He noted that the fluid injected ran into perfectly definite walled channels and that there was no extravasation until the pressure was too great, when the walls of the ducts would suddenly and explosively burst and the fluid would then pass into the meshes of the connective tissue. His conclusion was that the lymphatic ducts in the skin of the embryo pigs are closed ducts.

Inasmuch as in this communication the lymphatic system of the mammal will be traced in its development up to the stage represented in the frog, it will be necessary to keep in mind the amphibian lymphatic system. In the frog the large subcutaneous lymph sacs communicate by ducts with four lymph hearts or sacs, two in the neck and two in the inguinal region. From these sacs, ducts empty into the veins in four places, two in the neck at the junction of the subclavian and cardinal veins, and two in the inguinal region, where the femoral and sciatic veins join to enter the Wolffian body as the renal portal system. There are no valves except where the ducts enter the veins and there are no lymph glands.

[6] Ranvier: Comptes Rendus, Tome 121, 1895, p. 1106.

Up to the time when the pig embryo reaches the fish stage, that is to say, when the four visceral arches are plainly seen (see Keibel's Normaltafeln. 1. Das Schwein., Fig. 19), there are no lymphatics. This is true of embryos up to 14 mm. long, which corresponds to a human embryo of about five weeks. There are, however, in these early stages certain areas in which loose connective tissue, bounded by zones of denser tissue, forms channels of least resistance for fluid injected under pressure. For example, there is such an area around the central nervous system. If Prussian blue is injected just dorsal to the spinal cord near the tail it will not only outline the cord and the brain but will also surround the peripheral nerves as far as they have developed. Sections of such specimens, especially if thick, give deceptive pictures, for the blue granules lying in the meshes of the connective tissue look as if they were in definite ducts. This is, however, not the case, and though these wide intercellular spaces, being full of lymph, may be called lymph spaces, and may have an important relation to the nourishment of the nervous system, they are not a part of the lymphatic system. In sections this loose tissue often breaks away, especially around the nerves, and gives the false appearance of empty spaces. It is in a similar way possible to outline the Wolffian body at least in part by injection.

Another of these areas of loose tissue bounded by zones of denser tissue is found beneath the skin. If one injects Prussian blue into the tissue beneath the skin of the embryo pig, there will be at the point of injection a mass of the blue fluid from which straight, blunt processes reminding one of Budge's canals, run out often in parallel lines. These processes have no resemblance to the true lypmhatic ducts which lie at a more superficial level and can be injected over them, but are due simply to the separation of the connective-tissue cells and show that the intercellular spaces are lines of least resistance for fluid injected under pressure. These spaces are artificially widened by injection. The distance one can inject these spaces depends on the looseness of the connective tissue, and as the meshes of the connective tissue are widest around the central nervous system, it is here that one can inject the farthest.

Serial sections of several embryos of stages before the lymphatic system has begun, that is of pigs up to 14 mm. long, have been made. In these specimens the blood-vessels are injected and from a study of the sections it is clear that all the spaces in the body walls can be proven to be blood-vessels except the spaces between the individual cells. There are no spaces along the dorsal line in connection with the

cœlom, which could form a thoracic duct as Budge supposed. In this stage, before there are any lymphatics, many of the blood-vessels widen out into sinusoids instead of capillaries.[1] Some of these sinusoids are beneath the skin, and since they are many times the width of the capillaries, and since the endothelium which lines them is thinner than that of the capillaries, they look much like lymphatics. However, they are readily distinguished by their evident connection with the veins and by the fact that they contain blood.

The development of the lymphatic system was found in this way. We have an abundant supply of pig embryos at the Anatomical Laboratory. Every day large numbers of embryos of all sizes from under 10 mm. upwards are brought to the laboratory. Moreover, we are so near the abattoir that the embryos are often brought with the heart still beating. It is essential in injecting lymphatics to have fresh embryos, for after an embryo is once thoroughly cold it is impossible to get good injections. The best results are always obtained while the heart is still beating. The embryos must be injected immediately after removing them from the uterus and the skin must be kept moist while injecting.

I began with the study of the lymph glands and made the first injections of them by introducing the needle into the foot pads. If in pigs about 10 cm. long, the needle is inserted into the foot pads of the hind feet, ducts are readily injected which run to a gland in the inguinal region, while from the fore feet the ducts run up to a gland in the front of the neck. As younger pigs were taken, for example, below 6 cm., it became impossible to inject any ducts from the foot pads; and still younger, at 4 cm., it became impossible to inject the ducts subcutaneously in the side of the leg. In order to get younger stages of the glands it was thus necessary to inject nearer to them and it was found that in stages when no lymphatics could be injected in the legs they could still be injected with ease in the body wall. These ducts in the body wall run to two other glands, one over the crest of the ileum and one in the posterior part of the neck.

These injections gave the first idea of the gradual growth of the lymphatic system from the centre; because at a certain stage when lymph ducts could always be injected in the body wall, none were ever injected in the feet or legs; that is to say, the legs had not yet received

[1] Minot: On a hitherto unrecognized form of blood circulation without capillaries in the organs of vertebrata. Proceedings of the Boston Society of Natural History, Vol. XXIX, No. 10, April, 1900, pp. 185-215.

lymphatics. From this time on my attention was turned to the development of the ducts rather than of the glands, because I was passing to stages before the lymph glands were formed.

At this time, following the suggestion of Budge's theory of the thoracic duct developing from spaces behind the aorta, a needle was introduced into the side of the neck of a very young embryo, and passed behind the heart. The injection obtained proved to be venous, but by taking larger embryos lymph ducts began to radiate out from the point of injection; for example, in a pig 18 mm. long a few ducts could be injected just at the point of puncture. For this injection the needle was introduced straight inward at a point midway between the ear and the upper border of the arm. At this stage, while it was always possible to inject the small tuft of ducts at the neck, it was never possible to inject ducts in any other part of the skin. Taking a stage a little larger, a wider area or zone of lymphatics could be injected from the same point in the neck, but none were injected in any other place in the skin until the pig was 3 cm. long, when the ducts could be injected just at a point over the crest of the ileum. Taking a stage still larger a wider injection could be made from both of these two points in the skin, one at the neck and one over the crest of the ileum. The zone that could be injected at each stage was both definite and constant and an increase of the pressure simply ruptured the ducts at their tips instead of injecting them farther. Moreover, at any point within a zone, ducts could always be injected subcutaneously in fresh specimens, while at all points beyond these zones they could never be injected.

Thus two points had been discovered as the result of many injections, from which the superficial lymphatics spread or radiated out to cover the skin of the body and head. At a little later stage two other points were found from which the ducts grew to the legs, one of these being in the front of the neck, the other being in the inguinal region. A large number of injections were made at the two primary points until a very complete series of the zones possible to inject at each stage was obtained. This series included the stages from the time when the ducts can just be injected at the side of the neck in a pig 18 mm. long, up to the time when the ducts from the two points of radiation have met and anastomosed over the side of the body of a pig 5.5 cm. long.

The early stages of this development have been embodied in a diagram or composite picture, Fig. 1. The picture includes pigs of four different lengths, 1.8 cm., 2 cm., 3 cm., and 4 cm. Later stages are omitted to avoid confusion. The injections for the diagram were all

made from two points, marked a for the neck and c over the crest of the ileum. The letters are used to mark the area which can be injected in a pig of a given size. For example, c corresponds to a pig 3 cm. long and shows that the ducts are just beginning over the crest of the ileum, while from the neck they have already grown over the head and thorax, and over the face either side of the eye.

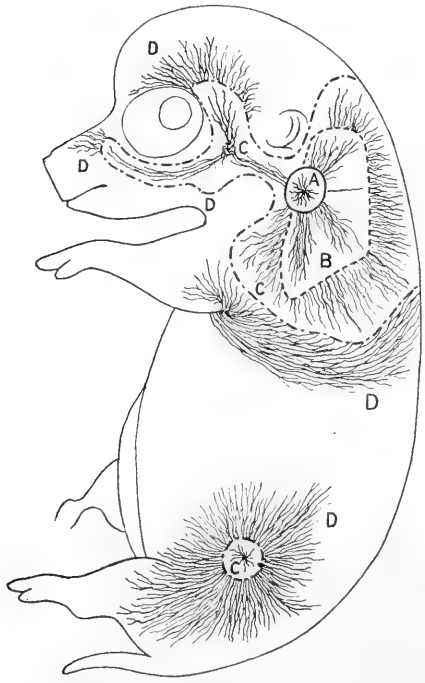

FIG. 1. Composite picture of the spreading of the superficial lymphatics in the embryo pig. A, area of lymphatics in a pig 18 mm. long; b, area in a pig 2 cm. long; c, area in a pig 3 cm. long; d, area in a pig 4 cm. long.

The areas without any ducts in the diagram represent the areas which have not received lymphatics in a pig 4 cm. long. In a pig 5.5 cm. long the two systems of ducts shown in the diagram have met and anastomosed over the body wall, and ducts have grown down the legs nearly to the feet, but there are still areas of the skin which have not yet received lymphatics; for example, the top of the head, the foot pads and the tail. The details of how these areas receive lymphatics as well as the relation of these ducts to the glands that form subsequently are given later in order to relate them to the lymph hearts. At this time we will limit the attention to the study of the successive zones of lymphatics and their relation to the areas without lymphatics.

The method of obtaining these injections is important. In the neck it was found that the best way to obtain maximum injections was to introduce the needle perpendicular to the skin, and it appeared later that this is due to the fact that one often enters the cervical lymph heart, a large sac from which all the ducts radiate. The method used was as follows: A glass tube drawn out to a fine point, the size of which should vary with the size of the pig, is held in a firm clamp which can be moved in three directions by screws. The glass tube is connected by rubber tubing with a flask of Berlin blue or India ink, and this flask is again connected with a pressure flask. The pig is placed on the

stage of a dissecting microscope, just enough pressure is used so that the fluid will drop slowly and the needle is screwed down into the side of the neck half way between the ear and the upper border of the arm. If the pig is not longer than 3 cm., tie the umbilical cord so as to fill the veins with blood and then introduce the needle into the side of the neck to a point just outside the anterior cardinal vein. For the point of radiation of the ducts for the lower part of the body, namely, over the crest of the ileum subcutaneous rather than deep injection are better for, as will appear later, the posterior lymph hearts are situated very deep.

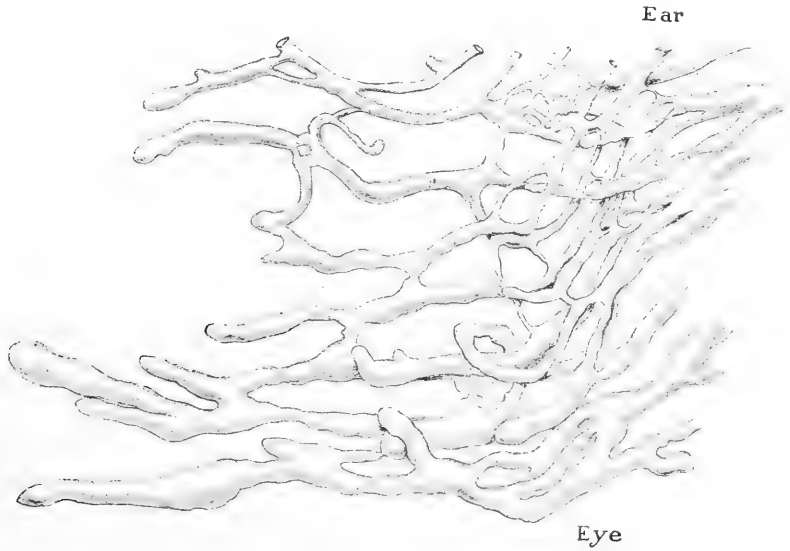

FIG. 2. Terminal lymphatics of the skin between the eye and the ear in a pig 5 cm. long. × 16.

It now became necessary to study more carefully these successive zones which had been injected, to prove that the ducts in the border of the zones were terminal and to note their relation to the skin areas not yet invaded by lymphatics. Figure 2 gives a picture of the border zone taken from the ducts injected over the side of the head between the eye and the ear in a pig 5 cm. long. This is a favorable place for study, for the ducts are larger and readily seen with the unaided eye. In general the ducts are the largest in pigs from 4 to 5 cm. long and they decrease in size as the pig grows larger. Other good places for studying the border zone are over the shoulders and over the side of the body. The ducts from the two sides of the neck meet and anasto-

mose between the scapulæ when the pig is 3 cm. long, so that the border zone is early obliterated here. Figure 2 shows that in the border zone the ducts grow out in advance of the plexus. In injecting, when the pressure is increased, these advance ducts always burst at the rounded tips, showing that they are the ends of the ducts. If now the ducts are just filled, care being taken not to burst them and each duct is touched just at the point where it leaves the plexus, with a small glass rod, it will be noted that the duct expands and contracts as the pressure is varied, or in other words, the wall is continuous and elastic. Occasionally it happens that one of these blunt ends, which may even be bulbous, is not really the end of a duct, for by a little pressure the injecting fluid can be forced out into a long thread-like process. That this is really a duct is shown by the fact that it can be filled and refilled by varying the pressure. These fine ducts or sprouts represent the process of growth at the border zone. In areas where the ducts are growing more rapidly than they do over the side of the head the terminal ducts are smaller than those shown in Fig. 2, and almost every one will have one or more long sprouts running out in advance.

It has already been said that no lymphatics have ever been injected in the areas beyond these zones of lymphatics in the different stages. Beside these injection experiments to prove that the ducts in the border zone are terminal and that no lymphatics can be injected beyond, complete serial sections have been made showing, first, the zone itself, with its rich capillary plexus, second, the zone of the growing tips, and third, the areas not yet invaded by lymphatics. These early ducts are so large that there is no mistaking them in sections; they are many times the size of blood capillaries. Uninjected specimens stained in acid fuchsin show them especially well in contrast with the blood capillaries.

I now considered the point proved that the lymphatics gradually invade the skin, for by making injections from either of the two radiating points in successive stages one can inject a wider area as larger pigs are taken and around these areas there is always a border zone of terminal ducts, which burst at the tips if pressure is used. The tips of these ducts are growing points and often have sprouts running out from them and finally, beyond this zone, there are no lymphatics, as has been proven both by their absence in sections and by a large number of negative injection experiments.

The next step in the growth of the lymphatics was to find out how they reached the surface. This was studied first in the neck. The

lymph ducts in pigs 4 to 6 cm. long were injected and the specimens were dissected so as to follow the ducts to the vein. When, however, the injection had gone over into the veins extensively the lymph ducts could not be distinguished from them. To overcome this difficulty the veins were filled with cinnabar gelatine and then the lymphatics were injected with a small amount of fluid, just enough to enter the vein. It is easy to see when the Berlin blue enters the subclavian vein, for the vein lies so near the surface. These specimens showed that the duct accompanies the anterior cardinal vein. In embryos between 18 mm. and 2.5 cm. long, if one injects subcutaneously in the side of the neck, small ducts pass inward and open into a large sac just external to the cardinal vein. The sac when injected is easily seen from the surface. Figure 3 is a section through it in an embryo 2.5 cm. long. This sac, which corresponds to the anterior lymph heart of the frog, is the key to the study of the subcutaneous lymphatics for the anterior half of the body, for they all radiate from it. In serial sections of this stage, the duct from the sac to the vein was traced to the junction of the anterior cardinal and subclavian veins.

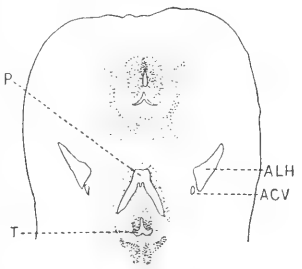

FIGURE 3. Transverse section through the neck of a pig 2.5 cm. long, showing the anterior lymph hearts, to be compared with Fig. 8. × 4.5. Acv, anterior cardinal vein; alh, anterior lymph heart; p, pharynx; t, trachea.

This method was sufficient as long as the sac could be injected from the surface, but in an embryo below 18 mm. in length it was again difficult to distinguish the lymphatics from the veins. To overcome this difficulty the veins were now injected. This can be done in three ways: First, if the umbilical cord is tied, there will be a natural injection of blood; second, if one injects into the liver the entire venous system will be injected, and third, in the younger embryos in which the liver is too small the Wolffian body answers the same purpose. By this method the sac which, being empty, contrasted with the injected vein was traced in serial sections in an embryo 15 mm. long.

Serial sections were now cut of embryos 12, 13 and 14 mm. long and showed no lymphatics whatever. However, in an embryo 14.5 cm. long a minute lymphatic sac connected with the vein was found. There are well marked differences in development between embryos 14, 14.5 and 15 mm. long. An embryo 15 mm. long shows the ear (See Keibel's Normaltafeln, No. 1. Das Schwein. Fig. 23), while an embryo of 14 mm. corresponds more to Keibel's Fig. 21, and shows indistinctly all four visceral arches. In the sections of the embryo 14 mm. long the

subclavian vein is represented only by a constriction on the side of the cardinal vein. At 14.5 mm. two visceral arches show on the surface and in sections the subclavian vein reaches the root of the arm bud. In this specimen the lymphatics are two small buds extending 228 μ from the vein. Figure 4 is the opening of this sac into the vein and shows that the entrance is guarded by a valve.

The valves which guard the openings of the lymphatic ducts into the veins have required considerable study. Serial sections of five different stages of embryos between 14.5 mm. and 3 cm. have been cut. The sections are stained on the slide with hæmatoxylin and a combination of eosin, 6 parts, aurantia, 1 part and orange G, 4 parts. In this stain the endothelium of the lymph ducts contrasts much better with the connective tissue than in sections stained with carmine. In all the sections of the openings of the lymphatics into the veins, the duct lies for some distance against the vein, the two being separated only by a double layer of endothelium, one for the vein and one for the lymph duct. Finally, in each series one can see that, just at the edge of the lymph duct, these two layers are continuous, see Fig. 4. But it was necessary to prove that this was actually an opening and for a long time I could not inject from the lymphatics to the veins in very young embryos. The reason for this is plain in Fig. 7. In injecting from the periphery one is injecting through small ducts into a large sac, and it is impossible to get pressure enough to force the fluid from the sac into its narrow efferent duct. One could easily fill the sac by puncturing it but the vein lies too near to be sure of puncturing the sac alone. However, by filling the sac carefully from the peripheral ducts one can then press gently with the finger against the neck and see that the fluid actually goes into the vein at the point of the junction of the subclavian and cardinal veins. It takes some pressure to open the valve, and the heart should be beating in the embryo used for the experiment. Having opened the valve, if serial sections are cut through it, the double fold of endothelium will be found a bit raised and smeared with India ink. India ink is a better fluid for these injections than Prussian blue. It runs farther and easier for its granules are smaller and they do not clump in the lymph. This has been done in embryos from 3.5 cm. down to 2.2 cm. long.

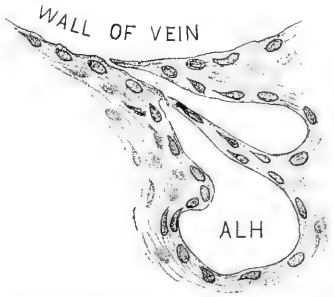

Fig. 4. Relation of the lymph duct to the cardinal vein in a pig 14.5 mm. long. Alh, anterior lymph heart. × 170.

In general, when a vein buds off from another vein, it grows out at nearly a right angle, while when a lymphatic buds from a vein, it grows at the smallest possible angle, or, in other words, it grows parallel to the vein. This difference in the direction of the growth of the lymphatic bud from the vein makes the valve at its orifice. (Fig. 4.) In the paper of Dr. W. G. MacCallum referred to above, he emphasizes the continuity of the endothelium in the lymphatic system and the fact that each endothelial cell comes from a preceding endothelial cell. This idea is here fully confirmed and carried a step farther, namely, that the endothelium of the lymphatic system buds off from the endo-

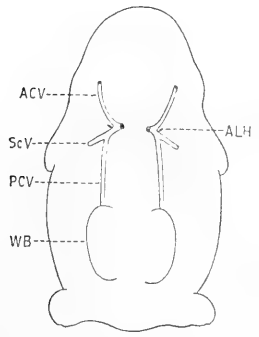

Fig. 5.

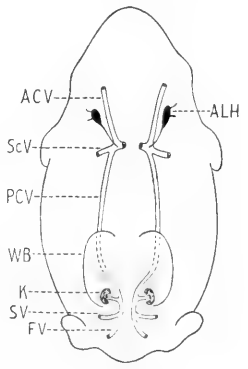

Fig. 6.

Fig. 5. Diagram of the lymphatic system in an embryo pig 14.5 cm. long. × 3. Acv, anterior cardinal vein; alh, anterior lymph heart; pcv, posterior cardinal vein; Wb, Wolffian body.

Fig. 6. Diagram of the lymphatic system in an embryo pig 15 mm. long. × 3. Alh, anterior lymph heart, fv, femoral vein; k, kidney; sv, sciatic vein.

thelium of the veins. In the stages considered in this paper none of the ducts nor sacs of the lymphatic system have any wall except a single layer of endothelial cells.

The proof that the lymphatic ducts bud off from the veins is as follows: It has been established that the ducts invade the skin from four points, two anterior and two posterior, and that the growth is from centre to periphery. Starting from the time when the ducts have completely covered the skin, every stage has been followed backward until the ducts are extremely small and extend only a short distance from the vein. In this stage the opening into the vein is just as perfect as in the later stages. Moreover, previous to the stage in which this bud connected with the vein is found, there is no trace of

a lymphatic duct or sac, as there would be if the sac formed first and subsequently joined the vein.

The general course of the early development of the lymphatic system in the embryo pig has been embodied in a series of diagrams, Figs. 5 to 11. They are made from actual injections of the lymphatics except in Figs. 5 and 6, for which the veins were injected. All of the specimens except the last two have been cut in complete serial sections. The lymphatics are drawn to scale except in the first one, in which the sac actually measures 228 μ and so had to be exaggerated a little to show in the diagram.

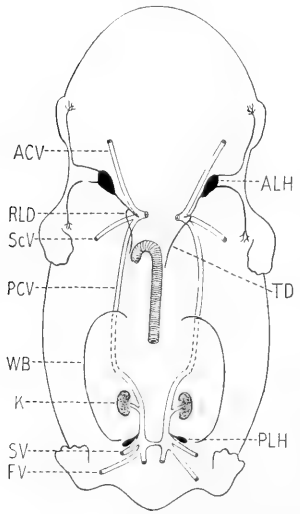

Fig. 7. Diagram of the lymphatic system of an embryo pig 2 cm. long. × 3. Alh, anterior lymph heart; plh, posterior lymph heart; rld, right lymphatic duct; td, thoracic duct.

The first diagram, Fig. 5, from an embryo 14.5 mm. long, shows the two lymphatic buds previously described, and their relation to the subclavian and cardinal veins. At this stage the posterior cardinal vein enters the Wolffian body. Figure 4 shows the sac and the valve which guards the entrance into the vein in this specimen.

The second diagram, Fig. 6, from an embryo 15 mm. long, shows the rapid growth of the ducts anteriorly. It is now evident that they open into a definite sac, corresponding to the cervical or anterior lymph heart of the frog. Small sprouts from this sac have started toward the skin. In an embryo 18 mm. long they have reached the skin and can be injected subcutaneously.

The third diagram, Fig. 7, is from an embryo 2 cm. long. The lymph heart is now large. Figure 3 shows its size in an embryo 2.5 cm. long. The ducts in the neck have now begun to spread in the skin, one tuft grows up behind the ear and another grows downward over the scapular area. This stage marks the beginning of the thoracic duct. From the two cervical lymphatic ducts which we have been following, two ducts start out, very near the opening into the vein, and grow posteriorly. The vagus nerve lies just behind the junction of the subclavian and cardinal veins and these two ducts follow the nerve on either side, and lie just internal to it. These two ducts are taken from sections and have not been injected at this stage.

The same specimen shows also the two posterior lymph hearts. In embryos from 15 to 19 mm. long there are no vessels in the lower part

of the body which are not definitely blood-vessels proved by injections. In the embryo 2 cm. long there are two lymphatic sacs connected with the posterior cardinal vein below the Wolffian body. At this point the sciatic and femoral veins unite in the posterior cardinal vein which enters the Wolffian body as the renal portal system. The two sacs, corresponding to the posterior lymph hearts of the frog, lie close to the vein, and have already sent sprouts out toward the skin. Their opening into the vein is also guarded by a valve so that they remain empty in a complete venous injection. Figure 12 is a section passing through the posterior lymph hearts in a pig 2.5 cm. long, and shows also the ducts reaching part way to the skin.

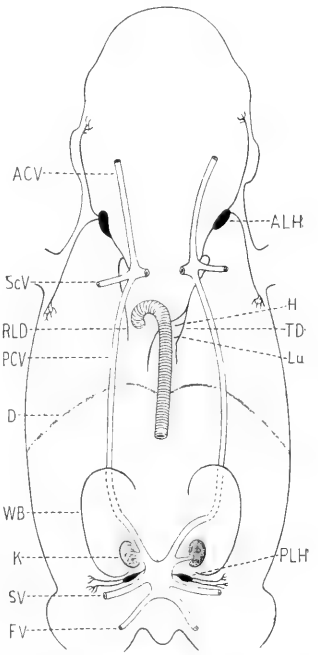

FIG. 8. Diagram of the lymphatic system in an embryo pig 2.7 cm. long. × 3. Alh, anterior lymph heart; d, diaphragm; h, ducts to the heart; lu, ducts to the lung.

The next stage, Fig. 8, is from a pig 2.7 cm. long. It shows a further development of the superficial lymphatics. At the same time it is the fundamental stage in the development of the thoracic duct and explains the asymmetry of the lymphatic system. The duct which grows downward on the left side meets the aorta and relying on its support grows more rapidly than the duct on the right side and so becomes the thoracic duct. It passes downward not only on the left side of the aorta but also sends a branch behind the aorta to pass downward on the right side. This may be called the right thoracic duct, to distinguish it from the right lymphatic duct. The duct which started out from the cervical duct on the right side remains as the right lymphatic duct, and in serial sections of a pig 3 cm. long has been traced to the root of the right lung. At this stage there are two buds starting from the left thoracic duct toward the heart and the lung. The thoracic ducts shown in this diagram as well as in all the succeeding ones, are from injections.

The method of injecting the thoracic duct is as follows. Occasionally, it is possible to inject the thoracic duct completely from the periphery in a pig about 4 cm. long, Fig. 10. In general, however, in

studying any area in the lymphatic system, one must inject close to the area. This is due to two facts: first, the ducts are very irregular and many exceedingly narrow ducts here and there check the flow of the fluid. These minute channels represent simply the process of growth and are not valves, for at this stage fluid always runs in both directions from the point of injection. Secondly, the lymph clumps the granules of the injecting mass and so closes the ducts. India ink will always run farther than Prussian blue. The study of the development of the thoracic duct depends on being able to inject it centrally.

The thoracic duct in these early stages lies actually in the edge of the wall of the aorta. The side of the thorax is cut away from the embryo, cutting the ribs close to their origin. The lower tip of the lung is freed and pushed aside, thus exposing the aorta and the posterior cardinal or azagos vein lying close beside it. The needle must now be introduced between the aorta and the vein and it must actually pick up the edge of the wall of the aorta. It is inserted just behind the intercostal branches of the aorta. In pigs 4 cm. long and upwards a fine hypodermic needle can be used, but in pigs smaller than 4 cm. a needle is too large and glass tubes drawn out to a fine point are necessary. At these stages, if one simply enters the loose connective tissue between the aorta and the vein without picking up the wall of the aorta, the injected fluid will extravasate. In older pigs, however, for example, over 20 cm. in length, the thoracic duct is separate from the aorta and can be injected by inserting the needle between the aorta and the vein. At this stage there is a plexus of lymphatic ducts in the aorta wall from which the thoracic duct can also be injected. The right lymphatic duct can be injected by dissecting the neck and introducing the needle behind the junction of the subclavian and cardinal veins.

Figure 9, from a pig 3 cm. long, shows the completion of the thoracic duct. In the thorax the two branches of the thoracic duct, lie either side of the aorta, between it and the azagos vein, but between the two kidneys they widen out to make a double receptaculum chyli and lie close together just behind, or dorsal to the aorta. The thoracic ducts on either side have now joined with the posterior lymph hearts and so the lower connection of the lymphatic system with the veins has been given up.

It will be noted that there has been a change in the venous system at the same time, for the femoral and sciatic veins now pass to the vena cava instead of following the old course into the Wolffian body as

the renal portal system. The area between the two kidneys is so rich in blood-vessels that a brief description of the veins will aid in locating the lymphatics in sections. This description is taken from a specimen prepared in this way. A fresh embryo of this stage, namely, 3 cm. long, was injected with carmine gelatine into the liver, and by this means the venous system was filled with red. The lymph ducts were then injected subcutaneously over the scapular area with India ink. The ink entered the vein and passed to the heart, which, as it was still beating, pumped the aorta full. A little of the black ink entered the vena cava, but not enough to obscure the picture. The embryo was hardened, cut in two parts in the median sagittal plain, cleared in creosote and mounted in balsam. In this specimen, the femoral and sciatic veins join to make a large median vein, the vena cava, which lies directly dorsal to the aorta opposite the posterior half of the Wolffian body. This median vein is connected by many branches with the posterior cardinal vein on the left side. Opposite the middle of the Wolffian body the vein is deflected to the right and comes to lie beside the aorta instead of behind it. The branch of the vena cava from the left kidney passes ventral to the aorta as in the adult. At this level the double receptaculum chyli is directly dorsal to the aorta. Opposite the anterior end of the Wolffian body the vena cava turns ventralward in the root of the mesentery and runs directly to the liver. Thus, in sections of this stage, opposite the posterior part of the Wolffian body it is the vena cava behind the aorta, while opposite the anterior half of the Wolffian body it is the double receptaculum chyli. This area shows especially well that in studying the lymphatic system either the veins or the lymphatics must be injected. The lymphatic

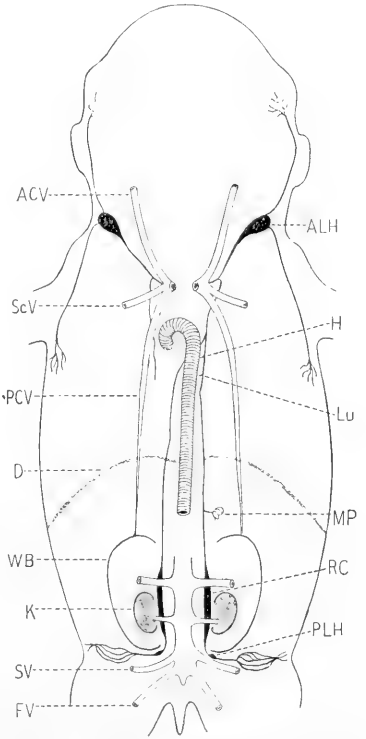

FIG. 9. Diagram of the lymphatic system in an embryo pig 3 cm. long. × 3. Alh, anterior lymph heart; h, ducts to the heart; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, posterior lymph heart; rc, receptaculum chyli.

ducts often have a rim of coagulated lymph which stains in eosin, and this together with the absence of blood cells aids in distinguishing them.

To return to the diagrams of the lymphatic system, Fig. 9 shows that the ducts from the posterior lymph hearts have just reached the skin. If a needle is inserted rather deeply over the crest of the ileum, the receptaculum chyli can be injected through them. The diagrams of the lymph hearts show why it is so much easier to inject the anterior one directly than the posterior one, namely, that the anterior one is superficial. To inject the posterior lymph heart directly, the abdominal wall must be opened and the needle inserted just below the kidney. There are so many veins in this region, however, that it is rare to get a pure lymphatic injection in this way. The receptaculum chyli is much more easily injected directly than the lymph heart.

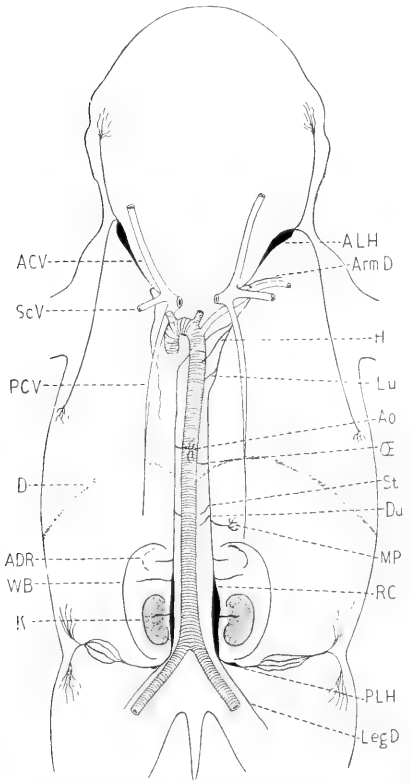

Fig. 10. Diagram of the lymphatic system in an embryo pig 4 cm. long. × 3. Adr, adrenal; alh, anterior lymph heart; ao, lymphatic plexus to the aorta wall; arm d, deep lymphatics to the arm; du, ducts to the duodenum; h, ducts to the heart; oe, ducts to the œsophagus; leg d, deep lymphatics to the leg; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, posterior lymph heart; rc, receptaculum chyli; st, ducts to the stomach.

At the stage of this diagram, Fig. 9, the lymph ducts to the heart have encircled the auricles. In an embryo 3.7 cm. long, lymphatics have been injected covering the inner surface of the left lung, while in serial sections of an embryo 3 cm. long there is a plexus of lymph ducts in the root of the mesentery, and the right lymphatic duct can be traced to the right lung. At this stage there has also been a new development in the superficial lymphatics at the neck. The diagram shows simply the two systems growing behind the ear and over the back. The ducts now grow both between the eye and the ear and in front of the eye. This shows in Fig. 1.

The last two diagrams show the further development both of the

thoracic duct and of the superficial lymphatics. In Fig. 10, from an embryo 4 cm. long, there is a plexus of ducts in the front wall of the aorta just above the diaphragm. Branches have been injected in this stage to the heart, lung, aortic wall, stomach, duodenum, adrenal, Wolffian body and kidney. At the same time ducts are growing along the subclavian and femoral arteries to make the deep lymphatics of the arm and leg. In Fig. 11, from an embryo 5.5 cm. long, there are additional branches to the reproductive organs. At the same time the superficial lymphatics have met and anastomosed over the sides of the body. The lymph hearts are now much smaller and are no longer definite sacs, but rather wide ducts.

From a study of the diagrams it will be plain that the superficial lymphatics starting from the veins primarily and from the four lymph hearts secondarily follow in general the course of the veins; in the neck the anterior cardinal, in the groin the sciatic. On the other hand, from the time the thoracic duct meets the aorta, the deep ducts grow along the arteries. This distinction was noted in the adult as far back as 1836 by Breschet.[8]

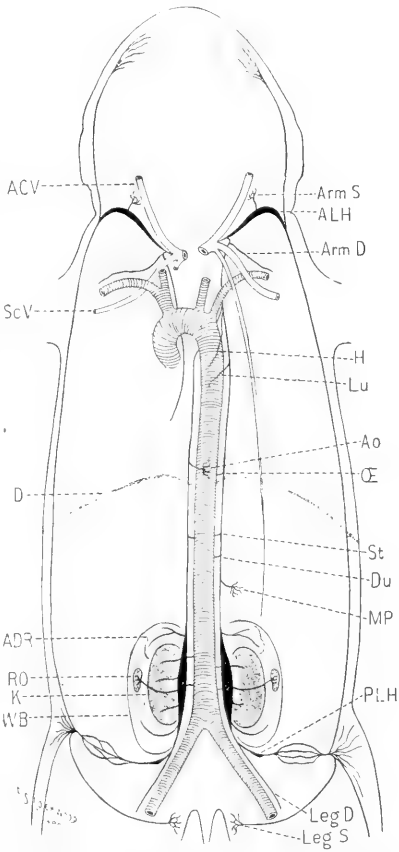

Fig. 11. Diagram of the lymphatic system in an embryo pig 5.5 cm. long. × 2. Alh, anterior lymph heart; arm d, deep lymphatics to the arm; arm s, superficial lymphatics to the arm; ao, plexus to the aorta wall; du, ducts to the duodenum; h, ducts to the heart; oe, ducts to the œsophagus; leg d, deep lymphatics to the leg; leg s, superficial lymphatics to the leg; lu, ducts to the lung; mp, ducts to the mesenteric plexus; plh, ducts to the posterior lymph heart; ro, ducts to the reproductive organs; st, ducts to the stomach wall.

The spread of the superficial lymphatics in embryos up to 4 cm. long is shown in Fig. 1. As has been said, all the ducts shown in the picture have been injected from two points, one in the neck marked a, which corresponds to the posi-

[8] Breschet: Le Systeme Lymphatique, Paris, 1836.

tion of the anterior lymph heart, and one over the crest of the ileum marked c. When the ducts first reach the skin in the side of the neck, one tuft grows behind the ear and the other grows over the scapular area. This is shown in the area marked b, corresponding to an embryo 2 cm. long. At the point of radiation of these two groups of ducts a plexus is formed which eventually becomes a lymph gland and corresponds to the group of glands in the posterior part of the neck of the adult.

From the anterior lymph heart another group of ducts grows to the angle of the jaw and there divides, one group growing over the head in front of the eye and the other growing between the eye and the ear. This second point of radiation, seen in a pig 3 cm. long in the area marked c in the diagram, is likewise a plexus, which becomes a lymph gland. A third point of radiation is made in the axilla for the ducts of the wall of the thorax. The ducts from this plexus are present in a pig 4 cm. long, as is shown in the area marked d. Finally, at the same stage, namely, in a pig 4 cm. long, a fourth point of radiation develops nearer the front of the neck for the superficial ducts of the arm and for the ducts over the front of the neck. This plexus has been injected in a pig 4 cm. long and by 4.5 cm. the ducts from it have reached the middle line of the neck in front and below the elbow on the surface of the arm. In Fig. 11 is seen the connection of this point of radiation with the anterior lymph heart; it is marked arm s.

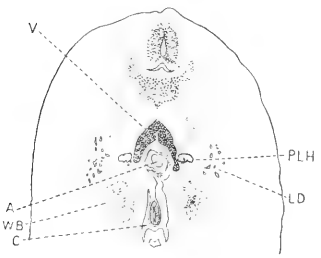

FIG. 12. Transverse section of an embryo pig 2.5 cm. long at the posterior end of the Wolffian body showing the posterior lymph hearts, to be compared with Fig. 8. × 5.5. A, aorta; c, cœlom; ld, lymphatic ducts growing to the surface; plh, posterior lymph heart; v, vein formed by the junction of the femoral and sciatic veins as shown in Fig. 8.

These four points of radiation, one in the posterior part of the neck or scapular, a second behind the angle of the jaw or maxillary, a third in the axilla or axillary, and a fourth in the anterior part of the neck or clavicular, are all connected in the depth with the anterior lymph heart, and can be injected from it. The branches that radiate out from the four plexuses anastomose so freely in the skin that they can also be injected subcutaneously. Certainly all the ducts in the anterior half of the body of a pig 5.5 cm. long can be injected by one puncture over the scapular area except possibly the ducts down the arm, which are too far away from the point of injection. In general, each one of these four points of radiation is a plexus which develops into a

lymph gland. Each gland then drains an area represented by the ducts that grew out from the plexus.

In the groin there are only two points of radiation for the superficial lymphatics. The first or primary one is over the crest of the ileum, and sends ducts over the back and sides of the body as far up as the axilla, and down over the hip. The ducts from this plexus do not grow to the middle line of the body in front, but anastomose freely with those of the other side across the back. The second centre, which comes a little later, is in the inguinal region and sends its ducts to the leg and to the ventral abdominal wall. In a pig 4.5 cm. long these ducts have grown to about the level of the umbilicus and extend part way down the leg. All the superficial lymphatics, after they have once covered the skin, anastomose freely; for example, one can inject in a pig 5.5 cm. long from the surface of the hip up to the axilla.

The deep lymphatics follow the arteries, which gives the key for the study of their development. By injecting along the aorta the ducts to the following organs have been injected: the heart, lung, œsophagus, stomach, duodenum, mesentery, adrenal, kidney, Wolffian body, reproductive organs, pancreas, spleen and liver. The ducts to the pancreas and spleen have only been injected in a pig 7.5 cm. long. I have, as yet, no early injection of the ducts of the liver. Fig. 11 shows the deep lymphatics of the arm and leg following the arteries. In a pig 7.5 cm. long the ducts following the umbilical arteries into the cord have been injected, that is, the lymphatics do not pass over to the umbilical cord from the abdominal wall but enter rather with the arteries. The right lymphatic duct has been injected in a pig about 10 cm. long and its branches pass to the heart and anastomose with those from the thoracic duct to that organ.

It has now been shown that the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction which the endothelial bud takes as it grows from the vein. In the ducts themselves there are no valves at first. From these two buds and later from two similar buds in the inguinal region ducts grow toward the skin and widen out to form four sacs or lymph hearts and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line following the aorta to make a thoracic duct from which the lymphatics grow to the various organs. Thus the ducts of the lymphatic system gradually invade the body, but there are

certain tissues which they never reach even in the adult, for example, the cornea and cartilage.

The development of the lymphatics has here been traced for the most part in pig embryos. We have, however, a rat embryo which corresponds with Fig. 6. The veins are filled with blood and so the lymph hearts are easy to find. Saxer[9] evidently has a cow's embryo 2.5 cm. long cut in serial sections, which shows the anterior lymph hearts. He describes the specimen as follows, that in the side of the neck are small lymphatic ducts which collect into two symmetrical cystic spaces, from which a duct narrows down rapidly and opens into the vein. Several of the human embryos of Professor Mall's collection have the veins well filled with blood, so that it has been possible to find the lymphatics in them and to confirm some of the steps of the development of the lymphatic system in the human embryo.

The lymphatic system, at the stage to which it has been traced in this work, represents about the stage of the adult frog. That is, it is a system of ducts without valves except at the openings into the veins and generally speaking, without glands. The plexuses which are to form the glands are present in many places.

The function of such a system is clearly suggested by a pathological specimen reported by Smith and Birmingham.[10] The specimen was of twins prematurely born, one of which was normal, while the other was so œdematous that it was simply a round ball. By good fortune the writers studied the lymphatic system in both fœtuses. In the normal one the thoracic duct and lymph glands were easily found, while in the œdematous one there was no trace of a thoracic duct and no lymph glands.

The lymphatic system is a modification of the circulatory system, dependent both in its origin and, in large measure, in its development on the blood-vessels. It returns to the vascular system the fluid exuded into the tissue spaces from the blood-vessels. Speaking more generally, it is a system of absorbents. The lymph glands which develop by the increase of connective tissue around plexuses of ducts come later; they occur only in birds and mammals and do not begin to develop in mammalian embryos until the ducts or capillaries they drain are well formed.

This study was begun at the suggestion of Professor Franklin P.

[9] Saxer: Anatomische Hefte 1, VI, 1896, S. 370.

[10] Smith and Birmingham: Absent thoracic duct causing œdema of a fœtus. Journal of Anatomy and Physiology, Vol. 23, 1889, p. 532.

Mall. It was his idea that by repeating and extending the experiments of Budge it might be possible to fill in the gap between the two lymphatic systems of Budge and clear up the subject of the origin of the lymphatic system, and this has been indeed the case. It is a pleasure to thank him for many suggestions and for his continued interest in the work. The opportunity for the work was made by a fellowship offered to the Johns Hopkins University by the Baltimore Association for the Promotion of University Education of Women. It is also a pleasure to express my gratitude to them.

THE DEVELOPMENT OF THE NOSE, AND OF THE PHARYNX AND ITS DERIVATIVES IN MAN.

BY

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WITH 13 TEXT FIGURES.

The presence of visceral folds homologous with the branchial arches of the fishes and lower vertebrates, the progressively changing circulatory system, the origin of the vertebrate mouth and its relation to the head and brain have combined to make the study of this region one of the most interesting and important divisions of mammalian embryology. In fact a knowledge of the development of this region not only aids us in understanding the adult anatomy but has been of the greatest service in giving us facts upon which to base definite ideas in regard to the evolution of these structures. There is scarcely a series of phenomena anywhere in the whole science of embryology that better illustrates the idea that "ontogeny repeats phylogeny" than the development of this region in the mammalia.

Most of the work done on this region has dealt with the visceral folds, the circulation and the origin of the thyroid and thymus glands, and the exact form of the pharyngeal cavities have had comparatively little attention, especially in man. The work of His[1] is especially valuable. Hammar[2] has also studied this region in man. He reconstructed the cavities and his work on the fate of the first branchial pocket and the origin of the Eustachian tube is complete and exhaustive. Piersol[3] has modeled the pharyngeal clefts in a complete series of rabbit embryos.

It seemed desirable to have a knowledge of the exact form of the

[1] His: Anatomie Menschlicher Embryonen. Leipzig, 1880. Also Beobachtungen zur Geschichte der Nasen- und Gaumenbildung beim menschlichen Embryo. Abhandl. der math.-phys. Classe der Königl. Gesell. der Wiss., Bd. xxvii, No. iii, 1901.

[2] J. Aug. Hammar: Studien über die Entwickelung des Vorderdarms und einiger angrenzenden Organe.

[3] G. A. Piersol: Ueber die Entwickelung der embryonalen Schlundspalten und ihre Derivate bei Säugethieren. Zeitschr. f. wiss. Zool., Bd. 47, 1888.

cavity of the human mouth and nose in the early stages of development, and hence at the suggestion of Dr. Mall I have made a series of models of the cavities of the mouth and nose of the valuable series of human embryos contained in his collection. The wax plate method of Born[4] was used throughout the work. The embryos used represent the first seven weeks of fœtal development. The magnifications were varied to suit the thickness and size of the sections of the different embryos used. In all the figures the cavities are represented as solid models and hence it is necessary to bear in mind that one is looking at a negative picture and not a positive one. The reverse is true, however, for the thyroid, thymus and salivary glands as these are solid objects and are so represented by the solid models. In nearly all of the models the mucous membrane was included. In the model of embryo XII and in one of the two made of embryo CLXIII only the cavity was modeled. For the model of embryo XII I am indebted to Dr. Mall. In making the illustrations to this paper an effort has been made to preserve enough of the embryo to make the relations of the model clear. At the same time details have been omitted in order to avoid making the whole appear too complicated and obscure the most important structures. For pictures of the entire embryos the reader is referred to the beautiful illustrations of Bardeen and Lewis,[5] where embryos CLXIII, CIX, and XXII are figured. These authors also refer to the articles previously written describing the embryos used for this paper. The following table will show the comparative size and age of the embryos used for these models:

Number of Embryo.	Length in mm.	Size of Ovum in mm.	Probable age.	Number of Embryo.	Length in mm.	Size of Ovum in mm.	Probable age.
			Weeks				Weeks
XII.....	2.1	18 x 18 x 18	2	CVI....	NB. 15.5 VB. 17		5½
II.....	NB.7 VB.6	25 x 25	4	CLXIV .	NB. 12 VB. 14	40 x 30 x 30	5½
CLXIII..	NB.9 VB.9	35 x 35 x 20	3½	XLIII ..	NB. 14 VB. 16		6
CIX....	NB. 10.5 VB. 11	30 x 30	5	XXII...	NB. 18 VB. 20	35 x 30 x 30	7
CLXXV.	NB. 13 VB. 13		5½				

[4] Morph. Jahrb., ii; Arch. f. mikr. Anat., xxii, p. 584. Also C. R. Bardeen, Johns Hopkins Hospital Bulletin, Vol. xii, April–May–June, 1901.

[5] Charles Russell Bardeen and Warren Harmon Lewis: Development of the Limbs, Body-wall and Back in Man. This Journal, Vol. i, No. 1, 1902.

Embryo XII.

This embryo is 2.1 mm. long and about 13 days old; and hence it represents a late two weeks' or early three weeks' embryo. The entire embryo has been modeled by Dr. Mall and I have used his model and the figure of it. In this embryo the pharynx is still in a very simple condition. The stomodæum has not yet broken through to form the mouth, the endodermal cavity shows Seesel's pocket and diverticula

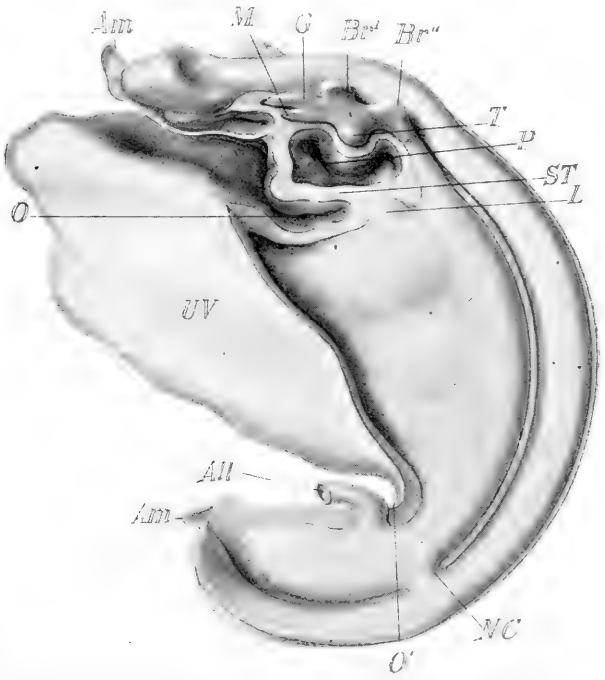

FIG. 1. Lateral view of the model of embryo XII (after Mall). Br.′ and Br.″, first and second visceral pouches; M, mouth just behind the stomodæum; L, rudiment of the liver; T, median thyroid rudiment.

corresponding to the 1st and 2nd visceral pouches. The general structures and relations can be seen in Fig. 1. To describe the model in detail from points of view not shown in the figure: Seen from above it presents a flattened slightly curved plate; the oral end resembling an ordinary cloverleaf in outline. The middle leaf represents Seesel's pocket, while the two lateral prominences correspond to the first pair of visceral pouches. Directly behind these are two smaller lateral swellings which fit into the 2nd pair of visceral pouches. These are

not exactly symmetrical as the right is caudal to the left one. Behind these distinct prominences two pairs of waves can be discovered in the outline of the edge of this flattened surface. These are the first traces of the developing 3rd and 4th visceral pouches. In the figure they have been almost completely lost. This surface is curved convexly between the prominences of the 2nd pair of visceral pouches and gradually flattens oralwards and caudalwards. Viewed from the side (Fig. 1) the same structures as noted from above, as well as some others on the caudal side of the plate, are visible. Directly under the prominence representing Seesel's pocket there is another of similar shape, but smaller, for the mouth. Just in front (ventral) of this the stomodæum appears as a decided pouch or pit.

On the under side of the model, on each side a ridge runs toward the middle line from the first pair of visceral pouches. At their point of junction in the middle line the median thyroid rudiment shows as a rounded eminence. This rudiment looked at directly from below is broader laterally than it is dorsoventrally.

The lack of bilateral symmetry of the pharynx and of the œsophagus is very marked. The position of the 2nd pair of visceral pouches has already been mentioned; in addition the left side of the œsophagus is thicker and heavier. At its caudal end it curves ventrally and ends in an eminence representing the rudiment of the liver. On the right side the œsophagus comes to a rounded edge. A cross-section would be wedge-shaped with the base of the wedge toward the left and the apex toward the right.

Embryo II.

This embryo has a vertex-breach length of 6 mm. and a neck-breach length of 7 mm. It is about 24 days old or, in round numbers, it is an embryo of the 4th week. The model of this region shows that marked changes occur in the period intervening between this and the preceding stage. The various regions are easily recognized. Seen from above this model is nearly an isosceles triangle. The base represents the mouth and hypophysis and the rounded apex the dorsal wall of the œsophagus. The sides of the triangle show three rounded swellings (see Fig. 3) corresponding to the 1st, 2nd and 3rd visceral pouches. The last named is the least prominent from this view since it is on a lower level and partly hidden by the projection of the 2nd. At this stage the 4th is well developed but much lower (caudal) and hidden from view by the 3rd.

Seen from the side, as in Fig. 2, the model bends almost at right

angles. At the bend there is a rounded prominence extending dorsally. Opposite to this prominence the 3rd and 4th visceral pouches project at right angles to the line of the œsophagus and trachea. The 2nd projects downward and is curved oralward. The 1st visceral pouch shows on its cephalic angle a greater degree of prominence than the other part of it. This is the first indication of the outgrowth of this part of the pouch to form the Eustachian tube. In this view there is

Fig. 2. Lateral view of the model of the pharynx of the model of embryo II. The embryo is represented as transparent. Magnified 25 diameters. Hyp., hypophysis; Oe., œsophagus; Tr., trachea, V. P.′, V. P.″, V. P.‴ and V. P.^{iv}, first, second, third and fourth visceral pouches.

much to suggest the model of the rabbit embryo shown by Piersol[6] in Fig. 8 of his paper.

From below the general shape is the same as when seen from above, but the picture is very much complicated by the projection of the visceral pouches, median, thyroid, rudiment, etc.

From the base of the prominence representing the 1st pouch a ridge runs backward on each side and terminates in the middle line. From the dorsal edge of this angle the median thyroid rudiment arises. It

[6] Loc. cit.

is still connected with the endoderm by a solid stalk (Fig. 3). Directly in front of this angle there is a rounded depression corresponding to the tuberculum impar. The walls of this depression unite in the middle line and form a ridge running oralwards in the middle line. On either side of this the depressions correspond to the mandibular processes. Directly back of the ridges connecting the first visceral pouches there are two deep rounded grooves; of which the two prominences of the 2nd visceral pouches form the dorsal boundaries. The model gradually narrows toward the œsophagus, and the two successive flat ridges, following one another and almost at right angles to the rest, represent the 3rd and 4th visceral pouches. The latter pair is not bilaterally symmetrical as the one on the right side is aboral to the one on the left side. This is not so decided in this embryo as in embryo XII just described. The left is also decidedly larger than the right one, and both show a tendency to become double at their extremities. This does not seem true in the next stage, represented by embryo CLXIII, although it is distinctly true for later stages. The disappearance and reappearance of this condition is not understood and in the absence of sufficient material it is suggested that there may be an individual variation in embryo CLXIII.

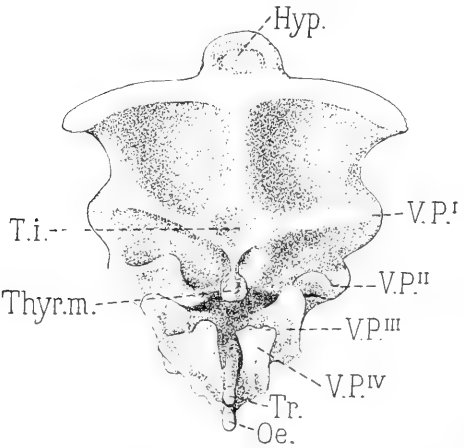

Fig. 3. Ventral view of the model of the pharynx of embryo II. Hyp., hypophysis; Oe., œsophagus; T. i., depression of the tuberculum impar; Thyr. m., median thyroid rudiment; Tr., trachea; V. P.′, V. P.″, V. P.‴ and V. P.ᴵᵛ, first, second, third and fourth visceral pouches.

In relation to the fate of the 2nd visceral pouch, His first stated that it formed the fossa of Rosenmüller and the tonsil. This is the description that is also given in text-books. Kastschenko[7] believes the fossa of Rosenmüller arises from a furrow between the inner border of the 2nd and 3rd arches. His,[8] in a recent article, agrees that it does not come from the 2nd visceral pouch.

In the middle line between the prominences of the 4th visceral pouches there is a thin flat projection, the beginning of the larynx.

[7] Kastschenko: Das Schicksal der embryonalen Schlundspalten bei Säugethieren. Arch. f. mikros. Anat., Bd 30.

[8] Loc. cit.

In this embryo the mouth is a transverse slit with only the hypophysis to break the lines. In none of my models does it appear as a five-sided object usually described since His first mentioned it as such in human embryos of the 3rd week. Hammar[9] also disagrees with His and finds it always as a transverse slit.

Fig. 4. Lateral view of the model of the pharynx of embryo CLXIII. Magnified 15 diameters. Hyp., hypophysis; N., outline of the side of the nasal depression; Oe., œsophagus; Thyr. m., median thyroid rudiment; Tr., trachea; V. P.′, V.P.″, V. P.‴ and V. P.ᴵᵛ, first, second, third and fourth visceral pouches; A. B., line of section seen, Fig. 6.

Embryo CLXIII.

This embryo has a vertex-breach of 9 mm. and a neck-breach 9 mm. in length. It is about 30 days old or an embryo of the early 5th week of development. Two models were made of the pharyngeal cavity of this embryo. The first represents the cavity simply, while the second one includes also the mucous membranes lining it. The description will apply to the model of the cavity alone and the second model will be

[9] Aug. Hammar: Notiz über die Entwicklung der Zunge und Mundspeicheldrüsen beim Menschen. Anat. Anz., Bd. XIX, 1901.

mentioned only where it is markedly different. The same structures are easily recognized here that were prominent in the embryo just described. However, the shape, size and relative proportions have changed markedly. Seen from the side (Fig. 4) another sharp bend has appeared at the oral end in addition to that at the aboral end already noted in embryo II. The part below this ventral (oral) end corresponds more nearly to the adult buccal cavity, especially the roof of it, than any structures that we have yet encountered. Just below this angle the hypophysis joins the mouth. From this angle the model runs dorsally in almost a straight line. This line is broken by

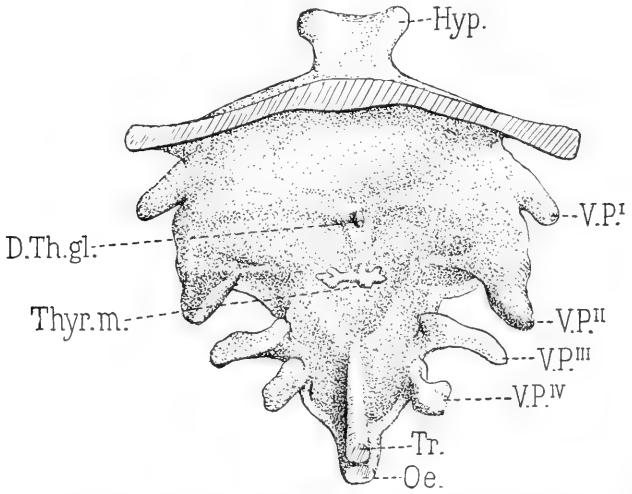

Fig. 5. Ventral view of the model of the pharynx of embryo CLXIII. D. Th. gl., ductus thyreoglossus; Hyp., hypophysis; Oe., œsophagus; Tr., trachea; V. P.′ V. P.″, V. P.‴ and V. P.ᴵᵛ, first, second, third and fourth visceral folds.

the 1st visceral pouch and the flattened process of the 2nd. Then there is another sharp turn caudalward with a slight dorsal prominence at the angle already mentioned in the description of embryo II. The line then bends slightly ventrally and shows the prominences of the 3rd and 4th visceral pouches.

Seen from above the model is roughly square. The hypophysis being at the ventral angle, the curved dorsal wall of the pharynx at the dorsal end and the two prominences of the 1st pair of visceral pouches at the angles of the square towards the sides of the embryo. Just behind these there is a deep rounded fossa and then a decided process projecting outward and backward, representing the 2nd pair of visceral

pouches. The 1st pair of visceral pouches are shown as prominent ridges running outward and backward. In the model of the cavity only, they project much farther free than in the one in which the model includes the mucous membrane. A para-sagittal section of this region of the embryo would show a relatively large opening, then a narrow one running dorsally and connecting with the cavity of the 2nd visceral pouch. This cavity (shown as a ridge on the model), representing the oral part of the 1st visceral pouch was called by Moldenhaur the sulcus tubo-tympanicus. Hammar uses the same term to mean the oral lengthening and development of the 1st visceral pouches as first used by Moldenhaur. Compare this structure in Figs. 2, 3, 4 and 5. Seen directly from behind (dorsally) all four pairs of visceral pouches are visible. The 1st pair running slightly upwards (cephalic). The 2nd pair show as two ridges flattened dorsoventrally and projecting downward. The 3rd pair stand out at right angles to the œsophagus and the cells have already begun to proliferate around it as a comparison of the two models will show. In the one in which the endoderm is included this pair of pouches is very much larger and exhibits processes which are not suggested by the model, showing only the cavity. The 4th pair are peculiarly shaped structures projecting at right angles to the œsophagus and then bending sharply dorsally on themselves. Their ends are enlarged and their outline is nearly triangular. They are relatively much larger in the model that includes the mucous membrane than in the one of the cavity only. In this model, however, they do not show a tendency to divide into two prominences as in embryo II or in others to be described later. Just beneath the 4th visceral pouch the whole pharynx constricts notably and marks off sharply the beginning of the œsophagus. See Fig. 4. A section of the pharynx taken at any point between the dorsal angle and the prominence of the 4th

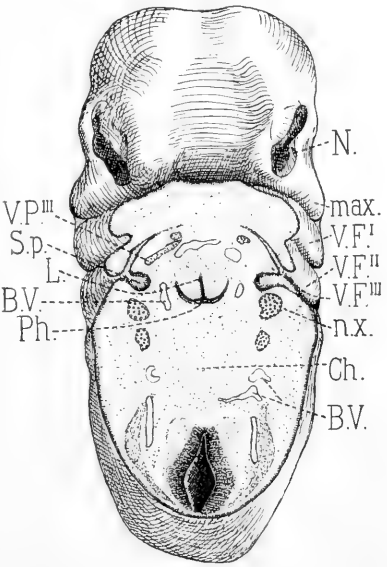

FIG. 6. View of reconstruction of embryo CLXIII from a ventral-caudal view. B. V., blood vessel; Ch., notochord; L., rudiment of larynx; Max., superior maxillary process; N., nasal fossa; N. x., vagus nerve; Ph., pharynx; S. p., sinus praecervicalis (His); V. F.′, V. F.″, V. F.‴, first, second and third visceral folds. Section on a line indicated by A. B., Fig. 4.

visceral pouch is crescentic in outline with a part projecting ventrally as shown in Fig. 5. Seen from the inside (caudal surface) the model shows the two ridges running from the prominences of the 1st visceral pocket toward the middle line. They are not so pronounced in the model of embryo II and are less prominent in the model of this embryo (CLXIII) that includes the mucous membrane than in the one where only the cavity is modeled. At the point of junction of these ridges in the middle line a small cylindrical projection marks the remnant of the thyreoglossal duct. The median thyroid rudiment has separated completely and sunken to the level of the 3rd visceral cleft. In front of these ridges the tuberculum impar has made a large depression. The median ridge seen in the model of embryo II has disappeared. Behind these ridges are two depressions showing the position of the two dorsal tongue rudiments. Behind (dorsalward) is still another depression situated in the median line the significance of which is not clear. Two ridges run caudalward and unite in the middle line to form a flattened body. This is the rudiment of the larynx.

Embryo CIX.

This embryo has a neck-breach length of 10.5 mm. and a vertex-breach of 11 mm. It is about 33 days old and so would be an embryo of the latter part of the fifth week. In spite of the apparently short time between this stage and the one just described very decided changes have occurred. The model includes the mucous membrane and is relatively large. In this model the angles are not so sharp and the various structures show a tendency to become rounded. Seen from the side as in Fig. 7 the same prominent bends are recognized but they are not so pronounced as in the model of embryo CLXIII. The ventral bend is larger and is joined to the nasal cavity as the illustration shows. The hypophysis is relatively higher and nearer the angle of the bend. The nasal cavities join the oral cavity at its edge and the posterior nares are in a position similar to the permanent condition in the frog. The prominence of the 1st visceral pocket is more complicated and the ridge running in from it toward the median line noted in the other two models is somewhat broken and irregular. The 2nd visceral pouch shows as a small knob projecting caudalward. The 3rd visceral pouch has disappeared as such and the 4th is prolonged into a hollow tube with two knobs on its caudal end. These will be described later as the lateral rudiments of the thyroid. At the point where they are attached the pharynx abruptly constricts into the œsophagus. The

œsophagus is shown as a rounded body and the cavity does not exhibit the typical Maltese cross shape as described by Minot.[10]

The trachea can be seen between the œsophagus and the 4th visceral

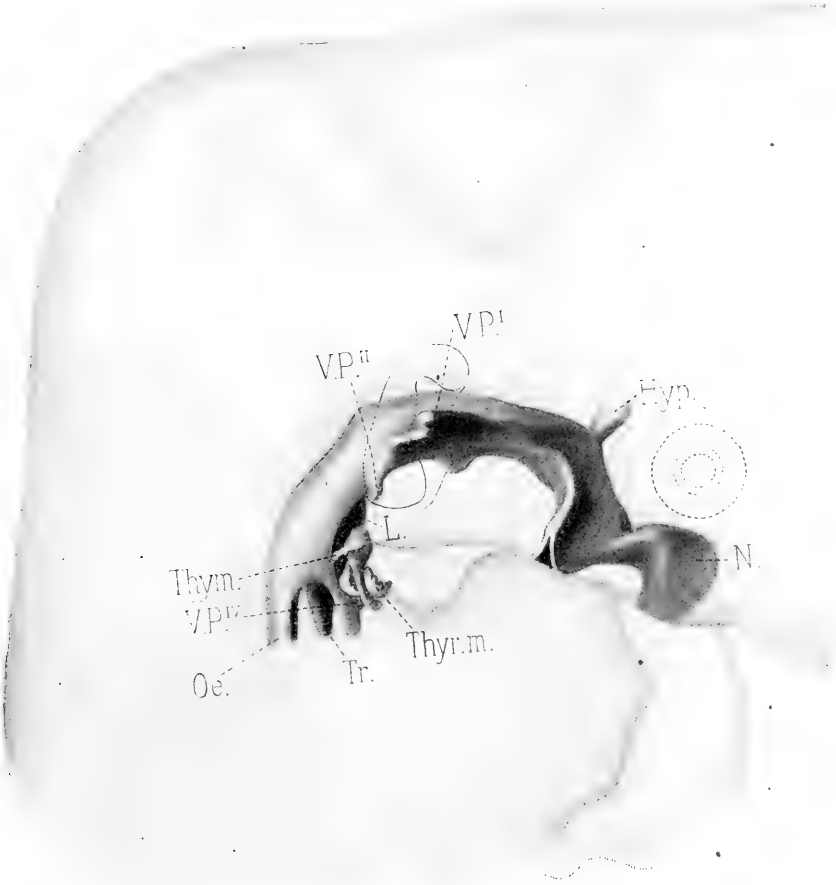

Fig. 7. Lateral view of the model of the pharynx of embryo CIX. Magnified 15 diameters. The embryo is represented as transparent. Hyp., hypophysis; L., rudiment of larynx; N., nasal cavity; Oe., œsophagus; Thym., thymus; Thyr. m., median thyroid rudiment; Tr., trachea; V. P.′, V. P.″ and V. P.ᴵᵛ, first, second and fourth visceral pouches.

pouch. At this level a section of it resembles a rounded bean with the hilum facing dorsally. Seen from above (cephalic) the model re-

[10] Charles Sedgwick Minot: Human Embryology. New York, 1892.

sembles the one just preceding in general outline except that the angles are decidedly rounded. The 1st visceral pouch appears as a prominent ridge (sulcus tubo-tympanicus) running outwardly and dorsally and ending in a rounded free prominence. The rounded fossa just back of it in embryo CLXIII has become here a flattened surface. Seen from the inside (caudal surface) there is a large central rounded cavity occupied by the tongue. A prominent U-shaped ridge separates it from a similar shaped groove, marking the position of the mandible. At the dorsal end of the tongue cavity a small conical projection marks the position of the thyreoglossal duct. Directly dorsal from that the larynx arises and the cavity shows as a T-shaped body. The top of the T facing ventrally. Caudalward the shape of the cavity changes gradually until the trachea is reached, when it has assumed the shape already described. Seen from the front (ventrally) the nasal cavities project outward from a large elevated fold in the middle line. They project away from the middle line at an angle of about 20°.

Embryo CLXXV.

This embryo has a length of neck-breach 13 mm., vertex-breach 13 mm. It is an embryo of the early part of the 6th week. This model also includes the mucous membrane and with a few interesting exceptions very much resembles the one just described. Looked at from the side the 1st visceral pouch has become somewhat complicated and altered. The most noticeable change being a prominent fold running from the 1st visceral pouch ventrally. The most striking difference noted from the side view is the lack of symmetry of the two sides. On the right side the hollow stalk connecting the lateral thyroid rudiment to the pharynx still persists. On the left side it has separated completely and the enlarged double end is fused with the now enlarged horseshoe-shaped median thyroid rudiment. On the right side the enlarged end of the lateral rudiment is in contact with the median rudiment but has *not* fused with it. Seen from above, this model differs from that of CIX chiefly in the increased rounding of the angles. The 1st visceral pouch, however, has changed and the fold running ventrally is much more prominent. This stage resembles the one figured by Piersol in Fig. 13 of his article already referred to. The hypophysis has just separated, although a slight thickening of epithelial elements mark its position on the model.

Seen from below (the inside) the model presents an even rounded hollow with the horseshoe-shaped groove surrounding it. This horse-

shoe-shaped depression marks the position of the mandible. The thyreoglossal duct is quite a large conical elevation. From its unequal sizes and characters in this series of models it seems to be a structure subject to considerable variation in its development. The larynx here is further developed and its cross-section is decidedly T-shaped. Just where the larynx arises there is a proliferation or overgrowth of epithelial cells. This is the beginning of a process which eventually closes the whole larynx for a period of fœtal life.

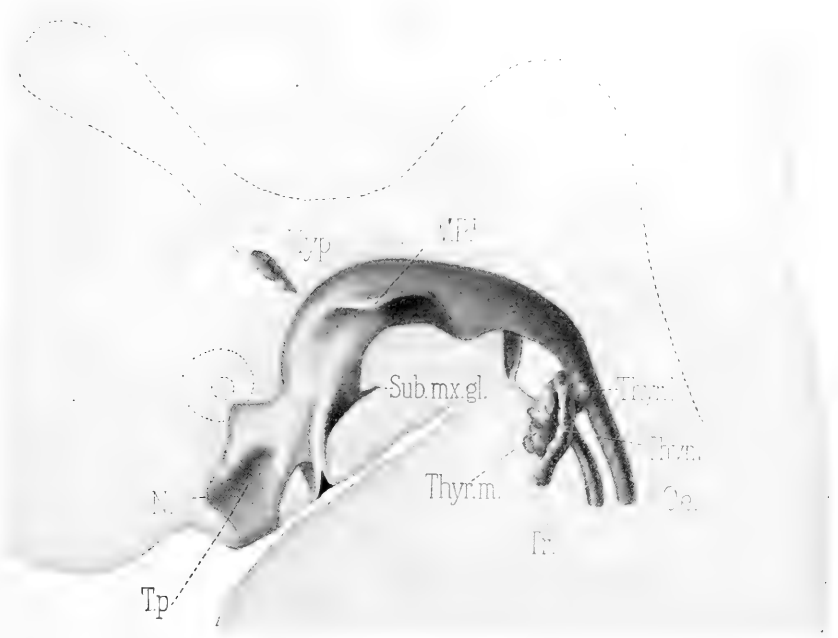

FIG. 8. Lateral view of the model of the nose and pharynx of embryo CXLIV. Magnified 15 diameters. Hyp., hypophysis; N., nose; Oe., œsophagus; Sub. mx. gl., rudiment of the submaxillary gland; Thym., thymus; Thyr. l., lateral thyroid rudiment; T. p., depression caused by the inferior turbinate process; Thyr. m., median thyroid rudiment; Tr., trachea; V. P.′, first visceral pouch.

Embryo CXLIV.

This embryo has a neck-breach length of 12 mm. and a vertex-breach of 14 mm. It is about 5½ weeks old. This model also includes the mucous membrane except the large endothelial plug which has nearly closed the larynx at its point of juncture with the pharynx. The closure is characteristic of this and later stages. In this embryo it is

still possible to trace the line between the two surfaces of the cells. Later even this is impossible.

Seen from the side, as in Fig. 8, this model presents a covered outline, almost U-shaped. The 1st visceral pouch (Eustachian tube) projects outward decidedly. It is elongated and thin rather than tubular. On its under side there is a ridge near the end and a complication of its outer end by a slight depression. What remains of the 4th visceral

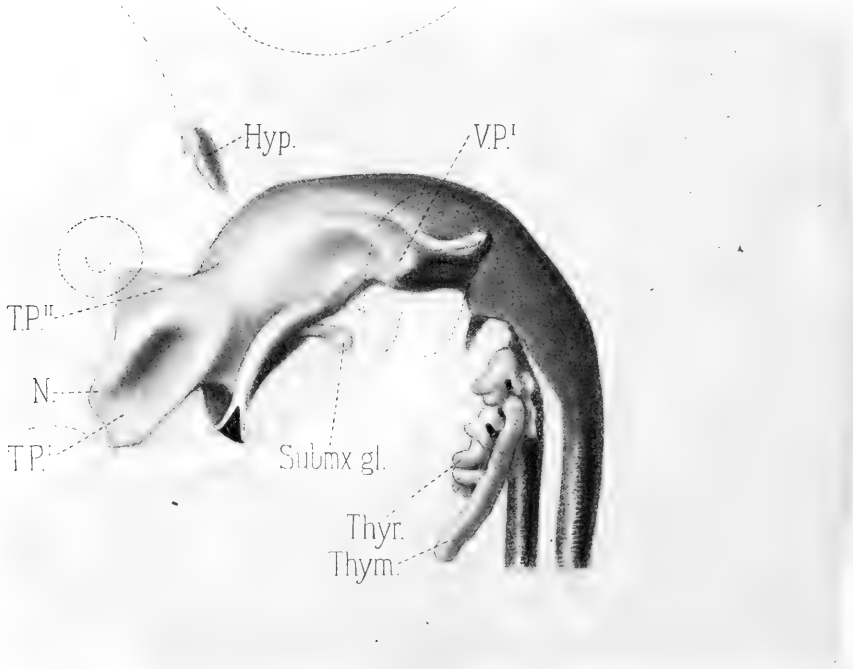

FIG. 9. Lateral view of the model of the nose and pharynx of embryo XLIII. Magnified 15 diameters. Hyp., hypophysis; N., nasal cavity; Sub. mx. gl., rudiment of submaxillary gland; Thym., thymus; Thyr., thyroid gland; T. p.′, depression caused by the inferior turbinate process; T. p.″, depression of the middle turbinate process; V. P., first visceral pouch.

pouch is completely cut off from the cavity of the pharynx, but the large lateral thyroid rudiments are still easily recognized as such, although both have now fused with the median rudiment.

Seen from above there is still a slight elevation where the hypophysis joined the mouth. The large ridge running between the nasal cavities is not so prominent and the cavities themselves are relatively much

nearer the middle line. From below, the cavity of the tongue is the largest feature. The larynx, as has already been stated, is plugged, but there is a large transverse projection corresponding to the arms of the T mentioned in describing the preceding models.

Embryo XLIII.

This embryo has a neck-breach length of 14 mm. and a vertex-breach of 16 mm. It is an embryo of the 6th week. This model also includes

FIG. 10. Lateral view of the model of the mouth and nose of embryo XXII. Magnified 10 diameters. N., nasal cavity; Oe., œsophagus; Sub. mx. gl., rudiment of the submaxillary gland; Thym., thymus; Tr., trachea; Thyr., thyroid; T. p.′, nferior turbinate process; T. p.″, middle turbinate process.

the epithelium except in the larynx where the plug is left out in order to show its position. This model is so like the preceding one just described that there is no need of a detailed description. The differences most striking in a side view of the model, as seen in Fig. 9, are the complete closure of the larynx by the epithelial plug; the lowered position of the thyroid and thymus rudiments; and the appearance of the rudiment of the submaxillary glands. The 1st visceral pouch (Eustachian tube) is bent upwards and the ridge running inward is still recognizable. In this view the nasal cavity is represented by an angular

body with a prominent horseshoe-shaped depression which was very noticeable in the model of embryo CXLIV.

Embryo XXII.

This embryo has a neck-breach length of 18 mm. and a vertex-breach 20 mm. It is an embryo of the 7th week. Seen from the side the nose is joined to the pharynx further dorsally and is relatively decidedly larger. The grooves representing the prominences of the jaws are more distinct and the space between them shows as a prominent flattened and slightly curved body (Fig. 10). The Eustachian tube is more prominent and although still very much flattened has assumed proportions nearer those of the adult. It is relatively more cephalic than in the other models. The thyroid and thymus rudiments are relatively further caudalwards. The epithelial plug of the larynx has disappeared and left the opening free. The rudiment of the submaxillary gland (Sub. max. gl., Fig. 10) is lobulated and more complicated than in the model just described. Seen from above the main body of the model is oval with the larger end ventralwards. The Eustachian tubes project laterally and dorsally. Ventrally the nasal cavities have approached one another and, instead of slanting away from one another, tend to approach as they leave the main body of the model. Seen from below the model shows a large rounded cavity for the tongue with a notch in the ventral border for the frænum. All traces of the thyreoglossal duct as a free opening has disappeared. Taken as a whole, this model is decidedly more rounded and the proportions are nearer those of the adult than in any of the embryos studied.

The Nose and Mouth.

In His'[11] article on the formation of the nose he introduces the subject by saying that the mouth and nose come originally from four separate sources, viz.:—the two nasal pits, the stomodæum and the pharynx (Kopfdarm). In regard to the nose he says the nasal pits develop as separate pouches above the stomodæum and the story of their development is the story of the development of the frontal process.

In the model of embryo XII (a late two weeks' embryo) two of these structures are present, the stomodæum and pharynx, while the two nasal pits have as yet not made their appearance. In an embryo of the 4th week the stomodæum and pharynx have united and the nose is represented by two olfactory plates barely outlined in Fig. 2.

[11] Loc. cit.

It is only when an embryo of about 4½ weeks old (embryo CLXIII) is studied that the olfactory organ has taken on the form of pits. Here they are two bean-shaped cavities (see Figs. 4 and 6). The caudal end is enlarged and a shallow groove runs towards the mouth cavity. It is not yet connected with the mouth cavity so it is not shown in Fig. 4 which is drawn from the side. The ridge overlying it, however, is shown faintly outlined. At its outer angle the superior maxillary process stands out as a rounded eminence. Fig. 6 shows the frontal process and an idea can be gotten of the comparative distance of the olfactory pits from the middle line in an embryo of this stage. In an older stage (5 weeks), represented by the model of embryo CIX, the four parts mentioned by His as forming the nose and mouth have united. In Fig. 7, which shows this embryo from the side, the nasal cavity is represented by two flattened processes attached to the buccal cavity by two slender stalks. These meet the mouth at an angle; that is, they diverge slightly as they leave the mouth cavity. These two flattened surfaces bear suggestions of ridges which are developed in the later stages, the most prominent being a cephalic dorsal one running backward toward the mouth opening. Seen from the inside there is a decided prominence at the angle where the pedicle and main nasal cavity join one another. This is the beginning of Jacobson's organ.

In an embryo slightly older, such as we have in the model of embryo CVI (this model is not figured), the plates seen from the side are no longer flat and the ridges shown in embryo CIX have become developed to so much greater extent that they can be distinctly recognized. These probably mark the first divisions into ridges and furrows that mark the rudiments of the turbinate processes. On the inner side the surface is also flattened and there is a small rounded knob marking the position of Jacobson's organ as mentioned for embryo CIX. Considering this part of the nasal cavity as a whole, the model shows it to be decidedly three sided. A cross-section of it would represent a right angled triangle. The right angle being at the junction of the outer surface with the lower (caudal) surface. The inner surface slopes downward towards the middle line and completes the outline of the triangle. The anterior nares run up to join this structure as a rounded body and meet it at right angles to the remainder of the structure. The posterior nares are a little larger than the anterior and show on their outer dorsal surfaces ridges continuous with the ridges on the surface of the nasal cavity. The entire model looked at from above shows that there is no angle of divergence between the models of the nasal cavities. They meet the mouth at almost right

angles. In an embryo of the 6th week (about 5½ weeks old), such as represented by the model of embryo CXLIV, the nose seen from the side, as in Fig. 8, is a rather irregular six-sided figure in outline. The pedicle is relatively larger and the groove running down on its outer side divides it into a horseshoe-shaped body. The triangular-shaped outline previously mentioned has been almost entirely lost. The anterior nares meet the main opening of the olfactory cavity, not at a right angle but almost in a straight line. In the model of embryo XLIII of the 6th week, but still older than the preceding embryo, the nasal cavity is relatively larger and occupies more of the model. The same general shape is preserved, however, as can be seen by comparing Fig. 8 and Fig. 9. The posterior nares are wider and are closer to the Eustachian tube. The same horseshoe-shaped folds are also prominent. The angle at which the nasal processes meet the mouth is here reversed and instead of diverging they approach one another as they leave the mouth cavity. In the nose of the oldest embryo modeled, that of a 7 weeks' embryo (embryo XXII), the nasal cavity is relatively much increased in size and is not so angular. The relative size of the posterior nares has increased constantly in relation to the size of the anterior nares. This enlargement seems to be a progressive growth in a cephalic and dorsal direction, and the result is to bring the rudiment of the Eustachian tube and the nose closer and closer as development proceeds. The folds on the lateral surface (see Fig. 10) have changed somewhat, but the tendency toward the horseshoe shape described in the other models is still recognizable here. From the inside, Jacobson's organ appears as a small conical elevation looking dorsally, and it is located near the caudal angle of the juncture of the pedicle and the large nasal cavity. In this model the cavities are relatively nearer to one another than in any of the models described, and seen from above they tend to approach one another slightly as they leave the buccal cavity.

Salivary Glands.

In reference to the time of appearance of the rudiments of the various salivary glands there is a difference of opinion among the different authors. Some of this discrepancy is no doubt due to uncertainty in regard to the exact age of the different embryos studied by different observers. Chievitz[12] states that the submaxillary appears in an em-

[12] J. H. Chievitz: Beiträge zur Entwickelungsgeschichte der Speicheldrüsen. Arch. f. Anat. u. Physiol., Anat. Abth., 1885.

bryo of a neck-breach length of 14 mm. (six weeks). Hammar [13] found it in an embryo 13.2 mm. long and His [14] in one 13.8 mm. long. In regard to the parotid Hammar states that he has found it at the end of the first month and that it does not appear as a solid rounded rod as generally described but as a groove which eventually closes off and forms the duct of the gland. His states that it appears at $7\frac{1}{2}$ weeks and Chievitz found it at the end of the 8th week. Hammar found the sublingual at 9 weeks and Chievitz states that he observed it a little before the rudiment of the parotid appeared, which would mean early in the 8th week.

In this series of models the submaxillary gland first appears in that of embryo CXLIV, where it is simply a rounded rod of cells staining deeper than the surrounding tissues. This embryo has a neck-breach length of 14 mm. In the model of embryo XLIII it is quite a large rudiment, as seen in Fig. 9. It is a straight cylindrical shaft with a knob turned directly away from the middle line. It meets the mouth cavity at the angle where the tongue and the ridge separating it from the groove of the mandible meet. It is a solid object throughout. In the model of the oldest embryo studied, embryo XXII, it has grown larger and the bulbus end has grown into an oval-shaped body covered with rounded enlargements marking the future lobes of the gland. It is still solid and the connective tissue around it shows a slight condensation into a capsule. No other salivary gland was observed at this stage.

Thymus Gland.

Stieda in 1881 was the first to observe that the thymus gland originated from a visceral pouch. Then came Born's important contribution stating that it arises from entoderm. This has since been confirmed by Prenant,[15] Mall [16] and His.

Beard [17] worked on a complete series of Raja embryos where the thymus develops from the first four branchial clefts, while a rudimentary thymus makes its appearance on the fifth. In this case the rudi-

[13] Loc. cit.

[14] Loc. cit.

[15] A. Prenant: Contribution à l' étude du development organique et histologie du thymus, etc. La Cellule, 1894.

[16] F. P. Mall: The branchial clefts of the dog with a special reference to the origin of the thymus gland. Studies from the Biological Laboratory, Johns Hopkins University, Vol. 4, No. 4, 1888.

[17] J. Beard: The Development and Probable Function of the Thymus. Anat. Anz., Bd IX, 1894.

ments arise just at the junction between the "epiblast and hypoblast," and he suggests that some cells of both layers are included in the rudiments and that the two kinds have different fates; that in the adult the corpuscles of Hassal represent the remains of the epiblastic cells, while the lymphoid tissue is the transformation hypoblastic cells. From its point of origin he compares its function to that of the tonsil and thinks that it is an organ developed to guard the gills and pharyngeal region. Capobianco[18] believes the lymphoid tissues are cells which have immigrated into the primary rudiment and that the lymphatic structure of the thymus was acquired secondarily. Kastchenko,[19] who worked on pig embryos, described the various details of the development of the thymus with great care. He thought the greater part of it came from the epithelium of the 3rd visceral pouch but that part also came from the sinus precervicalis.

Harman[20] describes the condition found in two babies at term where there were a socia thymi cervicalis and a thymus accessorius present, He refers to the condition in the sheep, where he says the 3rd and 4th visceral pouches participate in the formation of the thymus, and suggests that where there is an accessory thymus in man there may have been a reversion and the 4th pouch had given rise to it. He quotes Sir Astley Cooper as saying that he has frequently observed that the cervical portion of the thymus is higher on the right than on the left side.

In the present study the model of embryo II shows the 3rd visceral pouch as a ridge with a ventral free end (V. P.‴, Fig. 2), but no differentiation of tissue to suggest a thymus. In the model of embryo CLXIII the prominent ridge has disappeared and the 3rd visceral pouch projects out directly from the pharynx. This pouch has a slightly enlarged end (V. P.″ in Figs. 4 and 5). In the next stage, in an embryo of about 4½ weeks of age, the thymus (Thym., Fig. 7) approaches very closely the condition figured by Born,[21] on page 297 (fig. d.), in which the thymus has completely lost its connection with the pharynx, and its original hollow is closed to a crescent-shaped opening that is

[18] Capobianco: Contribution a la morphol. du thymus. Arch. ital. de biol., XVII, 1892.

[19] Kastchenko: Das Schlundspaltensystem des Huhnchens. Arch. f. Anat. u. Phys., Anat. Abth., 1887.

[20] N. Bishop Harman: "Socia thymi cervicalis" and Thymus accessorius. The Journal of Anatomy and Physiology, vol. XXXVI, Pt. 1, 1901.

[21] Ueber die Derivate der embryonalen Schlundbogen. Archiv f. mikros. Anat., Bd. XXII, 1882.

quite characteristic and aids greatly in identifying the gland at this stage. It is a curved, elongated mass with an enlarged superior cephalic end. The lower and smaller end runs parallel with the thyroid rudiment until that bends abruptly and crosses the middle line. The two structures do not come into contact at any point, however.

In embryo CLXXV, which is only slightly older, practically the same condition prevails as in the model just described. The differences are that the enlarged head is relatively smaller and the general curve of the whole rudiment is not so pronounced, as shown in Fig. 12. The small process that projects dorsally and laterally is still seen.

In the model of embryo CXLIV of the sixth week the relative position of the thymus rudiment is lower and the division into different areas and parts has disappeared. The head is barely recognizable as a slight enlargement. They have approached the middle line at the aboral end, although they still do not meet. In the model of embryo XLIII the thymus is still lower so that now the thyroid rudiment is above even the head of the thymus. The whole rudiment projects beyond the thyroid and is nearer the middle line. In the model of the oldest embryo studied, that of an embryo of the 7th week (embryo XXII), the thymus rudiment has sunken relatively lower. It is in contact with the thyroid rudiment along half of its upper surface and the other end is free. In this lower free half the two rudiments approach one another and meet in the middle line where the ends are slightly swollen and bend ventrally. Apparently this is a beginning of the folding of the thymus rudiment found in the adult organ. In this model the thymus rudiment on the right side extends a little higher than on the left side. The same condition is true in that of embryo CXLIV. This will be referred to later in the discussion of the lack of bilateral symmetry in the development of the thyroid.

The Thyroid.

The thyroid gland has proven of great interest to morphologists on account of its origin and change of position and function. In the tunicates it secretes an adhesive material used in the capture of the food of the animal. In the higher mammals it has sunken to a lower functional plane and its function has proven to be one of the puzzles of the physiologist. Remak discovered that it was of entodermal origin. Stieda and Wolfler discovered the lateral rudiments, independently, at the same time. Its origin is ably dealt with in Born's [22]

[22] Loc. cit. Es ist also ohne Zweifel, dass der epitheliale Theil der Thyroidea durch Verschmelzung zweier ursprünglich räumlich getrennter und histologisch

article. I give his exact words below because they have served as a basis for our knowledge of the subject since they were written.

In this series of models the thyroid rudiment appears in the earliest one studied (embryo XII at the end of the 2nd week). In this embryo it is represented as a rounded eminence directly on the ridges uniting the first pair of visceral pouches. It is broader from side to side than it is ventro-dorsally, making its outline elliptical. This is the rudiment of the thyroid gland and the ductus thyreoglossus.

In the model of the fourth week embryo (Thyr. m., Fig. 3, embryo II), it is a solid structure arising from the dorsal part of the apex of the angle formed by the junction of the ridges running inward from the 1st pair of visceral pouches. It is just oral to the hollow space representing the inner of the 2nd visceral arches. Directly oral to it is a hollow space representing the tuberculum impar. The thyroid rudiment consists of a slender stalk surmounted by a knob facing aborally (dorsally also in this embryo). Its shape might be compared to a pipe with a short stem and the bowl facing the back. The 4th visceral pouch (V. P.^{IV}, Fig. 2) is a flattened object with the part attaching it to the pharynx slightly smaller than the distal end. It shows no thickening or development into the lateral thyroid rudiment.

In the next stage, as seen in embryo CLXIII, the thyreoglossal duct remains as a small conical eminence on the ridge connecting the first visceral pouches (see D. Th. gl., Fig. 5). It is at the junction of the tuberculum impar and the two dorsal rudiments of the tongue. The median thyroid rudiment has become entirely disconnected and sunken to the level of the 3rd visceral fold. It has spread out laterally and has a bilobed structure. The left lobe is decidedly the longer and approaches closer to the floor of the mouth than the right. The 4th visceral pouches (V. P.$_4^{IV}$, Figs. 4 and 5) appear as two ventral projections with enlarged knobs bent sharply dorsally. In this embryo they show no tendency to become bilobed, as is seen in embryo II and in later stages. It has already been suggested that this may represent an individual variation.

verschieden gebauter Bestandtheile hergestellt wird; der eine von diesen, der unpaare, wächst in der Medianlinie aus dem Epithel der Vereinigungsstelle der zweiten Kiemenbogen aus, derselbe nimmt frühzeitig die bekannte Netzstructur an, der andre paarige Theil wird durch zwei schlauchartige, ventrale Austülpungen der vierten Kiemenspalten, die etwas nach innen convergiren, dargestellt; letztere verlieren erst nach der Verschmelzung mit dem medianen Antheil, der sich allmählich nach hinten verschiebt, ihre einfache Schlauchform und wandeln sich in ein Netz von Zellbalken um.

In the model of embryo CIX the median thyroid rudiment is U-shaped with a more or less irregular outline. The right arm, however, is decidedly shorter than the left. The transverse part of the thyroid that runs across the middle line is on the level with the 4th visceral pouch. This part shows the usual network structure formed by cords of solid cells. The arms of the U are crescentic in cross-section with the hollow looking away from the middle line and embracing the thymus rudiment, although the two do not come in contact. The lateral rudiments are still connected with the pharynx. They consist of hollow tubes surmounted by solid expansions. These are unlike on the two sides. On the right side the ventral knob is smaller and less

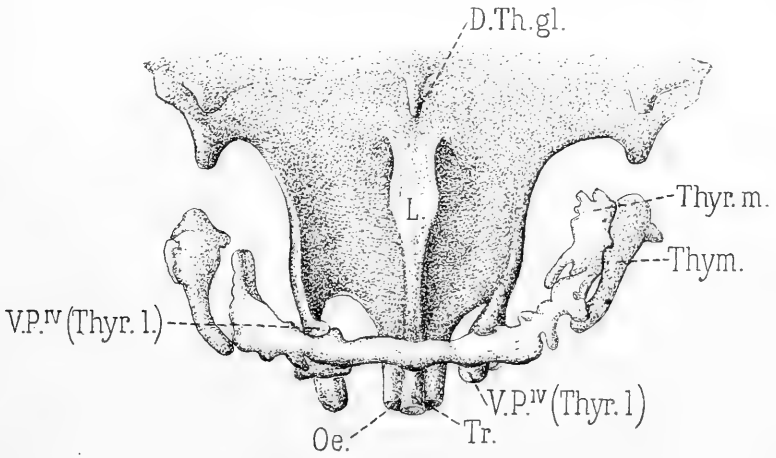

FIG. 11. Ventral view of the dorsal portion of the model of embryo CIX. D. Th. gl., ductus thyreoglossus. Oe., œsophagus; Thym., thymus; Thyr. m., median thyroid rudiment.

prominent than the dorsal, while on the left they are about equal in size and placed on the same level. This lack of bilateral symmetry seems an unimportant thing to note here, but that it is not simply an individual variation is shown by the fact that it occurs in the next stages.

In the model of embryo CLXXV the median U-shaped rudiment has sunken a little lower than in the preceding stage. The right limb is still smaller and not as long as the one on the left side. The lateral rudiments show a most interesting lack of bilateral symmetry (see Thyr. l., Fig. 12). On the right side the rudiment is still connected with the pharynx by a small hollow stalk. The ventral knob lies in contact with the middle of the right limb of the median rudiment but there is no

histological continuity. On the left side, on the contrary, the connection between the rudiment and the pharynx has disappeared. At a point corresponding to the stalk on the left side there is a short duct pointing toward a similar short duct on the lateral thyroid rudiment. The ventral knob of this rudiment is not only in contact with the median thyroid rudiment at a point corresponding to the one on the right, but a microscopical examination shows that there is actual continuity of the two structures. As in the embryo described just before this, the lateral thyroid rudiments are unlike on the two sides. On the right side the ventral knob is much smaller than the dorsal one, while on the left side it is the same size or a little larger. In both of these models the median thyroid rudiment shows a dorsal enlargement at the right-hand angle of the U.

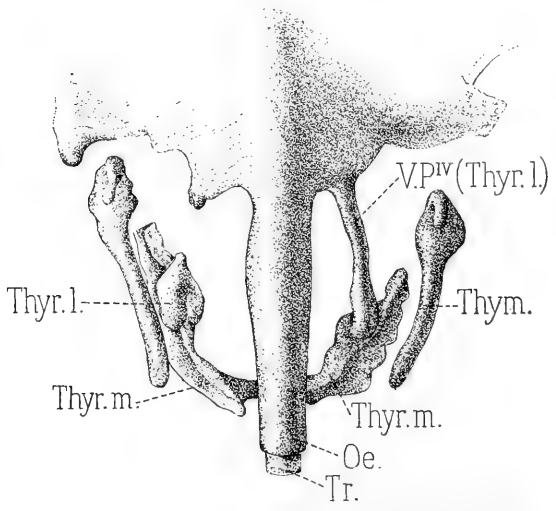

Fig. 12. Dorsal view of the dorsal portion of embryo CLXXV, showing the lack of symmetry of the lateral thyroid rudiments. Oe., œsophagus; Thym., thymus; Thyr. m., median thyroid rudiment; Thyr. l., lateral thyroid rudiment.

In the next stage, represented by embryo CXLIV, the median and lateral thyroid rudiments have united on both sides and all connection with the pharynx has been lost. They can still be recognized, however (Thyr. l., Fig. 8), as distinct rudiments. Under the microscope, however, there is structural continuity of the two parts.

In a later stage, in embryo XLIII, the structures have begun to assume more nearly their adult relations to one another. The lateral rudiments are a part of the median, and a few prominent lobules at the point of their union is all that is left to suggest their existence. The loop of the U is smaller while the limbs have enlarged and show lobulated structures suggestive of the condition in the adult gland.

In embryo XXII (7th week) the shape of the thyroid is almost that of the adult, the small isthmus connecting the two lateral lobes. The

two lateral lobes are pyriform in shape and attached to the isthmus at the smaller end.

Mall[23] has noted that in the chick and dog the branchial arches appear sooner on the left side than on the right and suggests that the development of the heart and bending of the head to the left is the cause of it. Attention has been already called to a similar condition in human embryos in this article, and it seems well to sum up the differences which seem distinctive of the two sides at the different stages.

The statement of Sir Astley Cooper in regard to the thymus usually being higher on the right side than on the left has been referred to before. This same condition has shown itself in these models. The thyroid, however, is the most marked example. In all of the models old enough to show a division of the median rudiment into the two lobes, the right one has been the shorter and the left one the longer, except in the embryo of the 7th week where the two were about equal in this respect. The lateral thyroid rudiments are unlike and their differences are most striking in embryo CLXXV where the right is still attached to the pharynx and the left one has lost its connection. The relative positions and differences of the visceral pouches of embryos XII and II have already been explained. Taking these differences together they show that in the human embryo the left side of the pharynx develops slightly more rapidly than the right side in the first few weeks of development. In the oldest embryo (embryo XXII of the 7th week) these differences seem to have disappeared and the two sides are symmetrical or very nearly so.

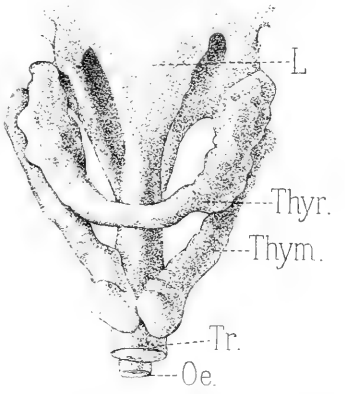

Fig. 13. Ventral view of the dorsal part of the model of embryo XXII. L., rudiment of larynx; Oe., œsophagus; Thym., thymus; Thyr., thyroid; Tr., trachea.

Summary.

The shape of the human pharynx changes from a more or less rounded cavity and one without many distinguishing characters, at the end of

[23] F. Mall: The branchial clefts of the dog with special reference to the origin of the thymus gland. Also, Development of the Eustachian tube, middle ear, tympanic membrane and meatus in the chick. Studies from the Biological Laboratory of the Johns Hopkins University, vol. 4, No. 4, 1888.

the 2nd week, to an angular, much differentiated cavity, during the 4th week. After that time it gradually loses its angularity, until it is a curved and well rounded cavity in the embryo of the 7th week.

The angle at the aboral end of the pharynx gradually shifts its position from a point opposite the 3rd visceral pouch in an embryo of the 3rd week, where it is most pronounced, until it is just oral to the 2nd visceral arch. As an angle it has completely disappeared in an embryo of the early part of the 6th week.

The Eustachian tube is the result of an extension of the cephalic angle and ridge of the 1st visceral pouch and the narrowing of the buccal cavity.

The sulcus tubo-tympanicus of Moldenhauer is a cephalic extension of the groove running toward the middle line from the 1st pharyngeal pouch. A part of it is converted into the mesial portion of the Eustachian tube by the conversion of the sulcus into a tube through the gradual narrowing of the buccal cavity.

The relative position of the posterior nares changes by gradually moving backward and becoming larger. The posterior nares and the Eustachian tube in the embryos of the first few weeks are widely separated but this shifting of the position of the posterior nares brings them into the relative positions which they occupy in the adult.

The turbinate processes show themselves as elevations quite early in the 6th week. The inferior is the most distinct. These gradually undergo differentiation and separation, but even in the 7th week they are still much unlike the adult condition.

In man the thyroid arises from the union of a median rudiment situated at the point of junction of the tuberculum impar and the two dorsal rudiments of the tongue with a paired rudiment arising as a differentiation of the lining of the 4th visceral pouch.

In man the rudiment of the thymus arises from the endothelium of the 3rd visceral pouch.

In the development of the pharynx the left side develops more rapidly than the right side, thus causing a lack of bilateral symmetry. This difference gradually decreases until by the 7th week the two sides have become about equalized. The cause of this difference is unknown but probably the bending of the head, as Mall suggests, may have some influence in determining it.

Note.—The original drawings to illustrate this article were made by Mr. George T. Kline and the reproduction of them superintended by Mr. F. S. Lockwood. I am greatly indebted to them for their care and interest in the work throughout.

A CASE OF HETEROTOPIA OF THE WHITE MATTER IN THE MEDULLA OBLONGATA.

BY

ALICE HAMILTON, M. D.

Chicago, Ill.

With 4 Text Figures.

In the medulla of a child of six years, dying of acute poliencephalomyelitis, an aberrant nerve tract was found, lying in the substantia reticularis grisea on the left side. The tract is visible to the naked eye for the greater part of its extent and shows under the microscope as a compact bundle of large medullated fibres, sharply marked off from the surrounding gray matter. Beginning at the upper end we find it emerging as a separate tract at the level of the lower end of the fourth nucleus, lying between the lemniscus medialis and the decussation of the brachium conjunctivum. It lies somewhat nearer the latter than the former but seems to belong to the lemniscus rather than to the brachium because the fibres are running longitudinally, as are the fibres of the lemniscus, while the fibres of the brachium conjunctivum are running across (Fig. 1). Going downward from this level the tract begins to lie more dorsally, and after the ventral part of the brachium conjunctivum has disappeared it lies in the substantia reticularis grisea a little nearer the lemniscus medialis than the fasciculus longitudinalis posterior, and nearer the mid-line than the outer edge (Figs. 2 and 3). At the upper level of the nucleus nervi facialis it is situated a little more mesially and ventrally, then it bends, runs transversely for a short distance until it reaches the trapezoid body, where it again takes a longitudinal course, lying now in between the fibres of the trapezoid, not far from the nucleus nervi facialis (Fig. 4). It disappears abruptly as if it had again bent upon itself, but the exact place of its termination could not be seen. It seems however most probable that it enters the nucleus nervi facialis.

To recapitulate: the tract arises from the lemniscus medialis, runs down in the substantia reticularis grisea, and enters the nucleus nervi facialis.

Instances of heterotopia of white matter in the central nervous

system are rare enough to be of interest no matter what their situation, but this bundle is perhaps unusually interesting inasmuch as it seems to form an additional proof of the truth of Hoche's researches on the

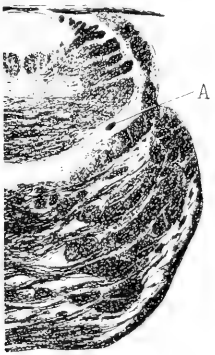

Fig. 1.

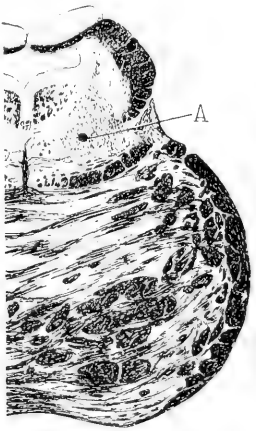

Fig. 2.

presence of centrifugal fibres in the lemniscus medialis. These fibres were described by Flechsig as receiving their medullary sheaths after birth, long after the larger number of fibres in the lemniscus medialis had become medullated, but our knowledge of their real nature is due to Hoche. In his study of two cases of descending degeneration from unilateral softening he found degenerated fibres, which high up in the pons were distinct from the lemniscus medialis, which then entered the lemniscus and passed down within it as far as the upper end of the nucleus nervi facialis, then becoming separated from the lemniscus these fibres passed, some to the nucleus of the same side, some to the nucleus of the opposite side. According to Hoche, these fibres as they leave the internal capsule come to lie in the peduncle lateral to the pyramidal tracts. They can be easily traced in his drawings made from Marchi sections, running into the lemniscus medialis and forming there, not a compact bundle, but many scattered bundles, corresponding exactly to the non-medullated fibres in the lemniscus of the new-born, as shown by Flechsig. Thus it seems that there are centrifugal fibres in the lemniscus medialis, some of which pass to the nucleus nervi facialis. In my case it would seem that some of these centrifugal fibres became separated on one side and ran as a distinct bundle, dorsal to the lemniscus, to terminate in the nucleus of the facial nerve on the same side. I can find no evidence, however, of a crossing of any of the fibres, indeed the bundle is throughout very compact.

There is, so far as I can find, no case of heterotopia exactly corresponding to this in the literature. I have been able to collect ten cases reported and one which has not yet been published. The usual situation of these aberrant bundles is in the dorsal part of the medulla,

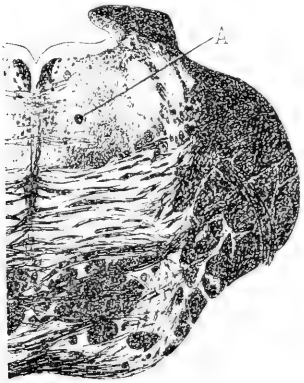

Fig. 3.

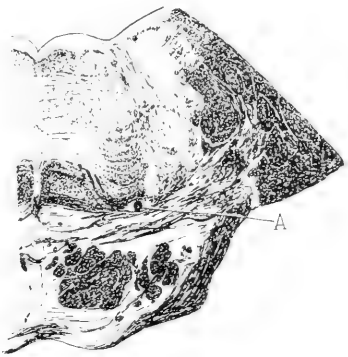

Fig. 4.

anterior and mesial to the substantia gelatinosa, extending from the lower end of the pyramidal decussation for a varying distance. Pick described a tract in this region and it has accordingly since then been termed Pick's bundle, although Henle had already described it but had failed to interpret it correctly. Pick traced it from the decussation to the level of the upper third of olives where it entered the corpus restiforme. He thought it identical with Henle's bundle, that is, one or two cylindrical cords with a diameter of 25 to 50 mm. situated in the posterior boundary of the reticular substance on one side, anterior to the head of the posterior horn. This bundle Henle had later on come to regard as the respiratory fasciculus and it remained for Pick to give the true interpretation and show that it was an anomalous tract running, according to his view, from the lateral columns to the corpus restiforme. Cramer's bundle also appeared at the level of the pyramidal decussation coming apparently from the lateral columns, the fibres radiating in the direction of the corpus restiforme; but their exact termination could not be made out. Cramer thought it probably identical with Pick's bundle. Schaffer's bundle, appearing at the upper end of the decussation, lay in the angle between the nucleus cuneatus and the nucleus gracilis, apparently came from the posterior, not the lateral columns, and ended in the corpus restiforme.

The anomalous tracts of Kronthal and Van Gieson were altogether

different from these. In Kronthal's case the fibres formed two well-defined bundles which appeared in the centre of the left nucleus nervi hypoglossi and after uniting to form a single cord lay near the raphé and not far from the floor of the ventricle. The fibres ended in a group of large cells, probably the nucleus centralis inferior. Van Gieson's is very briefly described as two bundles lying at the inner margin of the nucleus nervi hypoglossi. They were supposed to be either stray fibres from the fasciculus longitudinalis posterior or else association fibres from the cells of the nucleus nervi hypoglossi.

Heard reports three instances of heterotopia of the white matter, two of them being found in the same medulla. The first one, he thinks, corresponds to Pick's bundle, beginning at the lower end of the decussation of the pyramids, forming a distinct bundle which lay anterior and mesial to the substantia gelatinosa, and terminating above the upper end of the olives. The exact destination of this bundle could not be made out; perhaps, as in Pick's cases, it ended in the corpus restiforme, but more probably it passed up within the tegmentum. Heard's third bundle began in the same way as his first but could be traced much farther up. It lay at first midway between the tractus spinalis trigemini and the fasciculus solitarius, then between the nucleus nervi facialis and the second part of the root of this nerve, and was finally lost high up in the pons. Just where it ended could not be made out, but certainly not in the corpus restiforme. The second bundle described by Heard was found in the same medulla as his first one; it began higher up than the former two at about the level of the exit of the eighth nerve. Numbers of small fasciculi lay near the floor of the fourth ventricle on either side of the mid-line, just mesial to the nuclei funiculi teretis, both of which were greatly enlarged and sent fibres into the anomalous bundles. At the level of the nucleus nervi abducentis the fibres were gathered into one bundle which lay now a little to one side of the median line. Farther up the bundle occupied a position between the two fasciculi longitudinales posteriores and terminated in the nucleus centralis superior which, on this side, was much enlarged.

Obersteiner speaks of an aberrant bundle which was found by one of his students but not published. This is probably the one in the possession of Dr. Goodkind of this city, who was at this time working in Obersteiner's laboratory, and who courteously gave me the specimen to examine. The bundle in this instance is the same as that described as Pick's.

The last case reported is the most interesting, as it explains the real significance of most of the others, of all, that is, which correspond to

Pick's bundle. This is the case described by Hoche in the article already quoted. An aberrant bundle taking the course described by Pick with the exception of its termination in the corpus restiforme, was found on the degenerated side in Hoche's case and could therefore for the first time be accurately traced. As this bundle was partly degenerated it was at once proved to be a centrifugal tract instead of a centripetal, as it had formerly been held to be. Hoche traced it from the level of the upper end of the nucleus nervi facialis down to the pyramidal decussation, lying at first between the nucleus of this nerve and the fibres going to the knee, then nearer the ventricle, and finally, in the closed medulla, near the mesial end of the substantia gelatinosa, and terminating in the lateral pyramidal tract. He explains it as a premature decussation of some of the pyramidal fibres on one side, and evidently the explanation holds good for two of the bundles described by Heard, and probably for all the so-called Pick's bundles, which, it would seem, should be traced from above down, instead or from below up, probably becoming separated from the pyramidal fibres at varying levels in the different cases, but always terminating in the lateral tracts.

I wish to express my thanks to Dr. Hugh T. Patrick of this city, to whom I am indebted for the material of this case.

BIBLIOGRAPHY.

Pick.—Arch. f. Psych., 1890, p. 636.
Cramer.—Centralblatt f. allg. Path. u. path. Anat., 1890, No. 11.
Schaffer.—Neurolog. Centralblatt, 1890, p. 453.
Kronthal.—Neurolog. Centralblatt, 1890, p. 456.
Van Gieson.—N. Y. Med. Journal, 1892, p. 337.
Heard.—Trans. Am. Jour. Med. Sciences, 1894, p. 140.
Hoche.—Arch. f. Psych., 1898, p. 103.

PALMS AND SOLES.

BY

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From the Zoölogical Laboratory of Smith College.

WITH 21 TEXT FIGURES.

INTRODUCTION.

Through various recent papers by Galton, Klaatsch, Hepburn, myself[1] and others the epidermic surface of the human palms and soles has been subjected to renewed investigation and comparisons have been made with the condition found in other mammals, resulting in the establishment of an interesting morphological history, the main points of which may be stated as follows:

I. Pentadactylous quadrupedal mammals normally possess upon the volar surface of each paw ten raised pads or callosities which come in contact with the ground when walking and thus bear the weight of the body. Of these five belong to the terminal joints of the digits, while the remaining five belong to the palmar (or plantar) surface, and are distributed and designated as follows:

One *Thenar,* upon the thenar region at base of pollex.

One *Hypothenar,* upon the like named region along outer border.

Three *Palmar* (or *Plantar*), placed in a transverse row at base of digits, and corresponding to the intervals between digits 2-5.

II. This typical plan shows various modifications as, for example, the condition in the *Felidæ,* in which the three palmar (and plantar) pads fuse at an early embryonic stage into the single pad so conspicuous a characteristic upon the feet of that group.

III. In the lower Primates (e. g., *Innuus*) these pads have suffered much reduction in elevation and have lost their definite boundaries, but the areas corresponding to them are conspicuously marked by curious spiral or looped patterns seen in the papillary ridges of the epidermis, characteristic of this order, the cores or centres of these patterns corresponding to the centres of the lost pads. An extra 6th centre,

[1] My paper unfortunately covered much the same ground as one published by Hepburn a few months previous, and the conclusions reached, in so far as the two papers overlapped, were nearly identical. (See Article by Hepburn, Anat. Anz., Bd. XIII, No. 16.)

designated by me as the *accessory hypothenar,* appears in members of this order between the hypothenar and the 3rd palmar (or plantar) centres, the homologies of which are as yet uncertain. It is much smaller than the rest and may be merely a variant with but little morphological significance.

IV. In Arboreal Primates these patterns in the papillary ridges increase the tactile function by their varied direction, a function which is evidently of so great importance in an animal swinging from bough to bough that they are kept to a uniform standard through natural selection.

V. In man, and probably in some of the higher Anthropoids, the special phase of the tactile function furthered by this arrangement becomes of reduced importance, and thus the patterns are much less pronounced and are found in all stages of reduction, even to that of complete loss. As in all cases of vestigial organs, there is great individual variation, ranging from atavistic forms, in which several of the centres appear, to those in which the papillary ridges of the entire palmar and plantar surfaces run in wavy, approximately parallel lines, with no suggestion of loops or other definite patterns. Corresponding probably to the greater importance of the tactile function in the finger tips, the five apical patterns upon the balls of the digits show a lesser grade of this reduction, and, although often exhibiting reduction in the complexity of the pattern, seldom become as completely obliterated as in the palms and soles.

Our knowledge of the actual epidermic markings of the human palms and soles, a knowledge which is seen by the above to be of the highest importance, has been advanced, more than by anyone else, by Sir Francis Galton, who has published a series of investigations and observations covering a period of many years. Although he has treated his subject with a minuteness of detail which leaves nothing to be desired in the territory to which he has turned his attention, this territory has been almost entirely limited to a single small area, that of the volar surfaces of the terminal joints of the fingers, his "finger-tips." To the remainder of the palmar surface of the hand he devotes but little attention (five small figures of palms marked off into areas, together with three pages of text in "Finger Prints," pp. 54-56), and he dismisses the subject of the sole of the foot with the following extract (Ibid., p. 56): "The ridges on the feet and toes are less complex than those on the hands and digits, and are less serviceable for present purposes, though equally interesting to physiologists. [Does he not mean *morphologists?*] Having given but little attention to them myself, they will not be again referred to."

In his various works Galton has invented certain methods of study and investigation, and has established certain fundamental principles which are of application to the entire palmar and plantar surfaces, and he may thus be considered, after Malpighi and Purkinje, a pioneer and founder of the science here treated. His methods consist of various practical ways of printing and interpreting the course of the papillary ridges, and he has established two important principles; (1) that the papillary ridges do not change from birth to death but remain absolutely constant, even to the minutest details, and are capable of surviving a considerable mechanical injury, and (2) that the individual variation is so great that, even in the case of a single finger, there is no practical likelihood of its pattern being exactly duplicated by that of another individual, thus rendering these parts of the greatest value as a means of personal identification.

For several years past, since becoming interested in the preparation of a short paper upon comparisons between the epidermic "centres of disturbance" in the Primates and the pads of walking mammals (Anat. Anz., Bd. XIII, No. 8, 9, 1897) I have been engaged in the attempt to extend the work of Galton to the field left uninvestigated by him, namely to the palmar surface of the hand and to the entire volar surface of the foot, and I believe that the points thus far established are of sufficient general interest to allow publication as a report of progress, without waiting for more extended results which are dependent upon the obtaining of a very large amount of material and which will consume several years in elaboration. My method of studying the palms and soles has been exclusively by means of prints taken off by a slight variation of the method most favored by Galton; that of the use of printers' ink, applied with a rubber roller such as is employed in printing with the Edison mimeograph, and a moment's comparison of a real palm with a good print of the same will convince one of the superior utility of the latter. The only possible disadvantage is the reversal of right and left sides in the print, a condition to which one becomes accustomed as readily as to the reversal of the image in the compound microscope.

The main points of interest which have thus far resulted from the investigation may be summarized under the three following heads, which will be considered in order: (1) General morphology of the papillary ridges of both palms and soles; of interest both for comparison with the condition seen in other Primates and as furnishing a convenient source of material for the study of individual variation. (Whether these points will be found of ethnographic value in the study of different races is a point yet to be determined.) (2) A

STUDY OF THREE CASES OF SO-CALLED "IDENTICAL TWINS," which will be seen to have an important bearing upon the structure of the egg-nucleus and kindred biological problems; and (3) THE USE OF THE MAIN LINES OF THE PALMS AND SOLES FOR PERSONAL IDENTIFICATION, especially in criminal records; a method much more practical than the use of the finger-prints alone, as advocated by Galton, and more simple and rapid than the Bertillon system now in extensive use.

The following tabulation of the above results will present them more clearly and at the same time serve as a Table of Contents for the body of the paper:

I. *General Morphology.*—(a) Method of interpretation. (b) Nomenclature. (c) Variation in the lines and areas. (d) Occurrence of patterns. (e) Races and sexes.

II. *Palms and Soles in Identical Twins.*—(a) Actual condition as seen by study of three cases. (b) Theoretical bearing.

III. *Use as Means of Personal Identification.*—(a) Palms and soles vs. finger-tips. (b) Comparison with other systems. (c) Uses.

I. General Morphology.

a. Method of Interpretation.—In my paper of 1897, referred to in the introduction, I state among others the following conclusions. The italics, as used here, are for the purpose of the present paper, and were not so used in the original:

"III. In some Primates, including man, the mounds suffer a more or less complete reduction, so that often the epidermic figure, or 'centre,' is alone left to designate the spot. *In cases of extreme reduction, the epidermic centre may also disappear.*

"IV. In man the apical centres on the finger tips are fairly constant, that form designated by Galton as a "simple arch" being the most reduced. *The palmar, thenar, and hypothenar centres are of uncertain occurrence.*"

It was after writing this that I began the study of as many human palms and soles as possible, for the purpose of ascertaining the average degree of reduction of the "centres," and the amount of atavism, and for a time looked only for the centres themselves, i. e. for at least some loop or other disturbance of the papillary ridges marking the core of a pattern. In this way I found that such patterns were relatively a little more frequent in the sole than in the palm; that in the former, for example, a thenar pattern was almost constant; that, in the latter, one of the three palmar patterns was usually indicated, and so on; but

the method of investigation seemed in a way unscientific and unsatisfactory, and there was even some difficulty in attributing a centre to its proper place, owing to what seemed an excessive degree of development of one area at the expense of the other. In short, I experienced the difficulties common to all morphological work in which no basis of homology has been established, and I thus cast about for some accurate

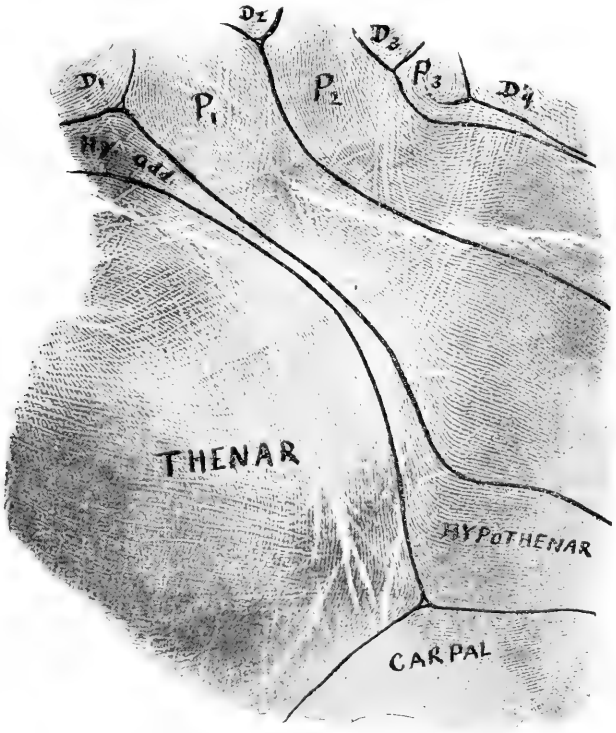

FIG. 1.—Right hand. Print and interpretation. Palmar areas open.[1]

means of dividing the surface under investigation into definite areas, *without the necessity of depending upon the presence of loops or other indications of patterns.* As I found patterns that had suffered all degrees of reduction even to almost complete suppression, it seemed

[1] EXPLANATION OF LETTERING.

Lines: p_1–p_4, palmar; do_1–do_4, outer digital; di_1–di_4, inner digital; Th, thenar; co, outer carpal; ci, inner carpal. Areas: P_1–P_3, palmar; D_1–D_4, digital; Th, thenar; H, hypothenar; H^1, hypothenar addition; C, carpal. Triradii: tr_1–tr_4, palmar; tr. c., carpal.

likely that areas in which the ridges run evenly and parallel must still have the same morphological value as though there were a bend or turn in one or more of the ridges and that the palms and soles should be studied, *not by searching for patterns, but by devising some accurate and scientific method of determining the boundaries of the areas corresponding morphologically to those in which the patterns had been located in the ancestral form.*

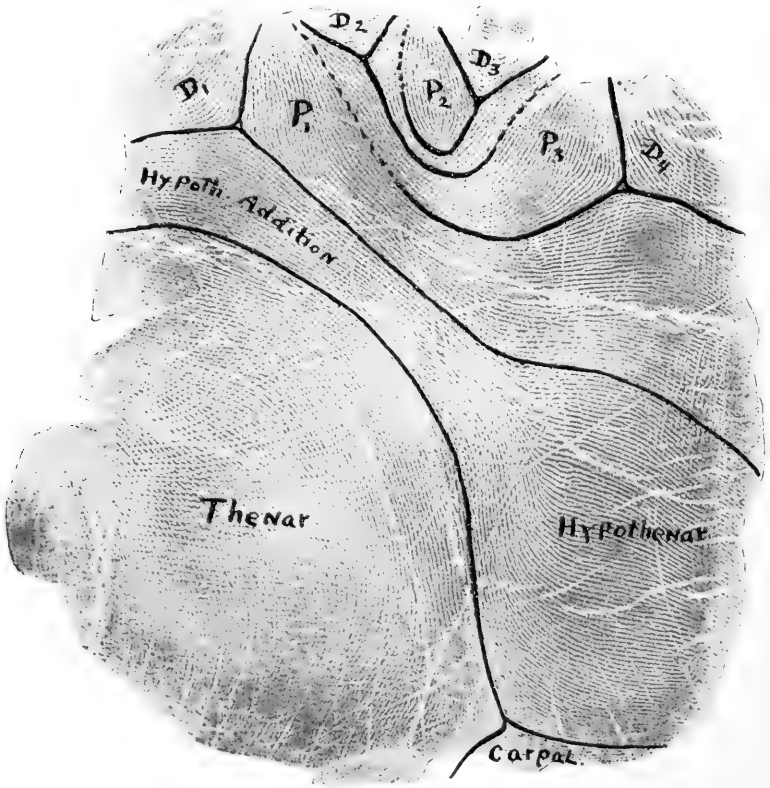

Fig. 2.—Right hand. Print and interpretation. Palmar areas divided.

With this end in view a comparative study was first made of several palms and a starting point for such a system was found in the discovery of four constant points, or "*triangular plots*" similar to those so named by Galton and used as the guiding points in his study of the finger-tips and in his method of interpretation. These four points, for which I prefer the name triradii to the more cumbersome term of Galton, are seen in figures 1 and 2, and appear in the majority of hands a short way

below the base of the fingers at the apices of four small, but well-marked triangular areas formed by the prolongation and intrusion of the papillary ridges of the fingers into the surface of the palm, the four *digital areas* ($D_1 - D_4$).

Each triradius is in the form of a minute triangle with its three angles prolonged, and, by continuing these angles, three lines are obtained in the form of a Y, of which the two upper run to the sides of the finger-base and bound each digital area, while the third runs down over the palm and forms one of a series of four *palmar lines* which mark off this region into three *palmar areas* (Figs. 1 and 2, P_1-P_3). The remaining surface includes the extensive *thenar* and *hypothenar areas*, with usually an extension of the latter, and the small and often obsolete *carpal area;* three territories which are usually located and bounded by lines extended from a *carpal triradius* at the base of the palm. In some cases, however, there is neither a definite carpal area nor a carpal triradius and there the line separating the thenar and hypothenar areas is indicated by a *parting* or separation of the ridges at the middle of the wrist.

b. Nomenclature.—That the lines and areas thus determined are of definite morphological value seems to me highly probable, and by the application of these methods to more than a hundred palms their constant nature becomes evident, although the actual modifications are, as might be expected, very numerous, and when the number of independently modifying concepts is taken into consideration it may well be seen that there is almost no possibility of two separate palms being identical even in the general plan. (See below, the paragraph on "Identical twins.")

The morphological elements of a normal human palm are thus seen to consist of *lines*, *areas*, and *triradii*, which may be conveniently tabulated as follows, each name being followed by a convenient abbreviation for use in the figures and to assist in the construction of descriptive formulæ. (See below, under "Personal identification.")

LINES.		AREAS.	
4 Palmar,	[$p_1 - p_4$]	3 Palmar	[$P_1 - P_3$]
8 Digital { 4 outer	[$do_1 - do_4$]	4 Digital	[$D_1 - D_4$]
8 Digital { 4 inner	[$di_1 - di_4$]	1 Thenar	[Th]
1 Thenar	[th]	1 Hypothenar	[H]
2 Carpal { outer	[co]	1 Hypothenar addition	[H_1]
2 Carpal { inner	[ci]	1 Carpal	[C]

TRIRADII.

4 Palmar [$tr_1 - tr_4$]
1 Carpal [tr. c]

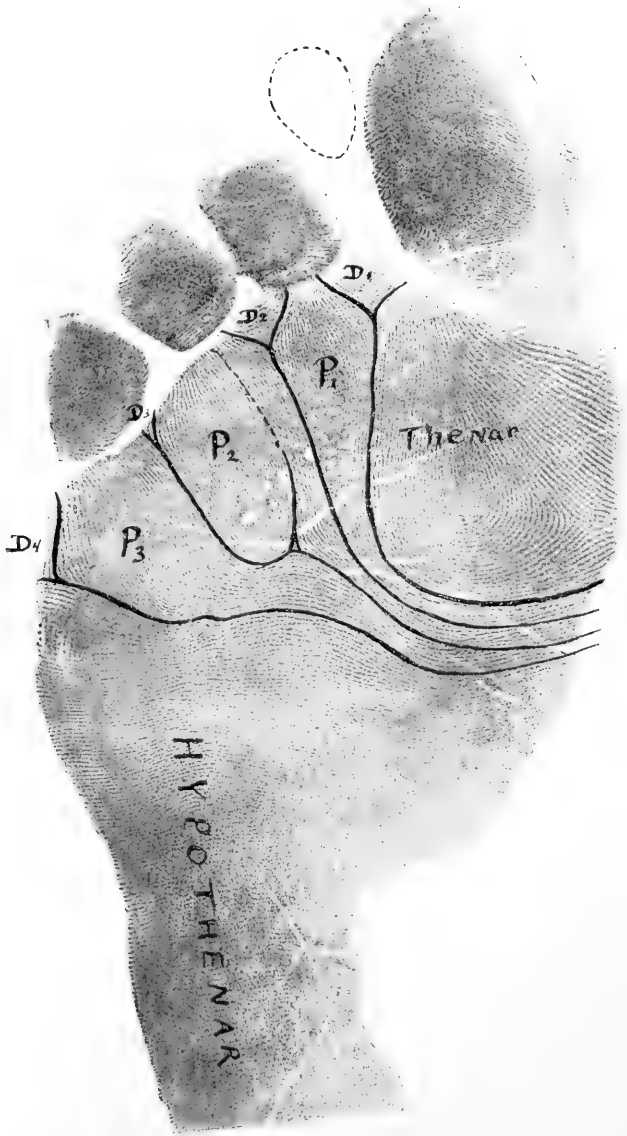

FIG. 6.—Left foot. Print and interpretation. First and third palmar areas open; second partly circumscribed by recurrence of third palmar line.

The morphology of the sole follows closely that of the palm and may be interpreted in the same way, as a comparison between Figs. 1 and 2 and Fig. 6 will show. In the sole, however, the general course of the ridges is from an outer distal to an inner proximal direction, the opposite to that of the palm, thus causing the plantar areas (the equivalent of the palmar of the hand), wherever they attain the margin, as in Fig. 6, *to open upon the inner, instead of the outer margin, and thus interpose themselves between the thenar and hypothenar areas, which are therefore not in contact with one another, as in the palm.* Other peculiarities characteristic of the sole are: (1) the enormous lengthening of the foot in the direction of the heel, an extent which seems to belong morphologically to the hypothenar area; (2) the more or less constant appearance of *accessory triradii* at the lower or proximal ends of the areas,

Fig. 3.

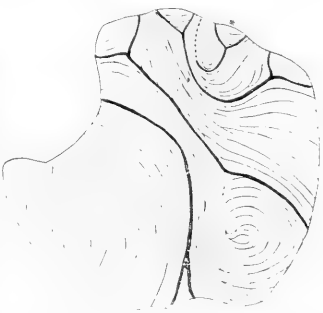

Fig. 4.

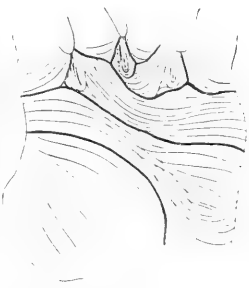

Fig. 5.

Fig. 3.—Right hand. Tracing. Third palmar area circumscribed. Third and fourth palmar lines confluent.

Fig. 4.—Right hand. Tracing. Second and fourth palmar lines confluent, circumscribing the second and third palmar areas.

Fig. 5.—Right hand. Tracing. Palmar lines as in Fig. 4. Three palmar areas and hypothenar provided with patterns.

the lines from which may or may not coincide with those drawn from the upper set; (3) an almost constant appearance of the thenar pattern, sometimes with one, sometimes with two triradii of its own, thus corresponding to the two forms of finger-tip patterns of Galton; (4) the greater frequency of appearance of plantar (= palmar) patterns, and (5) an occasional case of confluence between two adjacent digital areas. All of these peculiarities are atavistic and thus correspond to the greater conservatism in structure and in use shown by the foot in comparison with the hand.

I have thus far interpreted a somewhat smaller number of soles than of palms (76 soles, 100 palms), but thus far I have found no deviation

too great to be interpreted morphologically by the above rules. As atavism is here often more marked, so also is the reduction of atavistic peculiarities occasionally more pronounced, and thus *the range of variation seems greater in the sole than in the palm.*

c. Variation in Lines and Areas.—After the collection and interpretation of 100 palms, I spent some time in the attempt to tabulate the principal variations occurring in the morphological elements and, although at times the task seemed endless, there was finally evolved a definite system dealing with a comparatively few primary forms to which all the variations could be referred. I am reserving the complete exposition of this for a later publication, but the scope of the present paper will allow me to indicate a few of the more fundamental variations. In regard, first, to palmar and plantar areas, the most usual human type is that of the *open area*, or one which attains the margin of the surface under consideration, its boundary lines terminating in the normal skin of the sides and dorsum of the member. As pointed out above, such areas open in the case of the palm upon the outer and, in that of the sole, upon the inner margins, and this type may be characteristic of all the palmar (or plantar) areas [Figs. 1 and 7], of two of them, [Fig. 3], or of but one, [Figs. 4, 5 and 8], the one most frequently open being the first in the palm and the third in the sole, owing to the reverse direction of the ridges. Fig. 9 shows the very unusual case of a plantar area (P_1) opening to the outer margin.

In contrast to the above, all other palmar (and plantar) areas are *closed*, but they may be completely *circumscribed* [as in Fig. 3, P_3 or Fig. 8, P_1 and P_2], or may be partially or wholly *confluent* with another area [Fig. 4, P_2 with P_3; Fig. 2, P_1 with P_3; Figs. 10 and 11, P_1 with P_3]. When an area is traversed by one of the palmar lines, thus making it partially confluent with another or partially open [Fig. 2, P_1] it may be termed a *divided area*, and instances sometimes occur in which all three palmar areas are divided, thus rendering the mutual relations extremely complex [Fig. 2]. Lastly, one of the palmar (or plantar) areas may be reduced [Figs. 10 and 11, P_2], or in some instances may become completely wanting, in which case its two limiting triradii become almost or entirely fused into one.

The relation in the hand of thenar, hypothenar and carpal areas becomes conditioned by the relationship of the carpal triradius, or by its absence. In 50 hands examined with reference to this, 32 possessed a carpal triradius, and of the remainder, 10 possessed at the wrist a definite "parting" of the ridges which indicated the starting point of the thenar line, 5 possessed a "seam," i. e. a slight wave in the successive

ridges, indicating the boundary between thenar and hypothenar areas, while 3 were set down as "indefinite," a condition due in part to incomplete printing.

d. Occurrence of Patterns.—The occurrence and relative frequency of patterns upon the palm may be seen from the following table, compiled from 100 hands (50 individuals). In this any definite looping or ridges, even when confined to a single ridge, is considered a pattern. It may be noted that while the same number of monkey palms would give 600 patterns, the total number occurring here is 131, indicating the degree

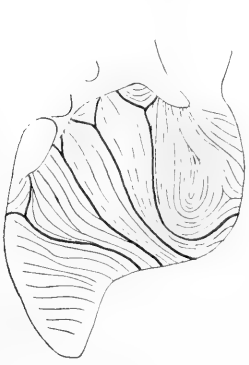

FIG. 7.

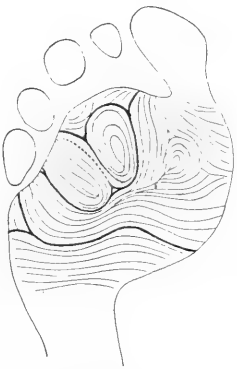

FIG. 8.

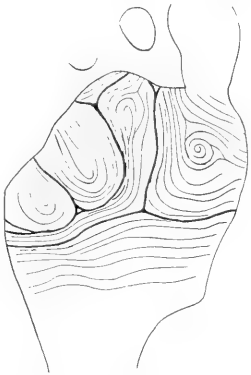

FIG. 9.

FIG. 7.—Left foot. Tracing. Three palmar areas open without patterns.

FIG. 8.—Left foot. Tracing. First and third palmar lines confluent. First and second palmar areas circumscribed, containing patterns. Third palmar area open.

FIG. 9.—Left foot. Tracing. Second, third and fourth palmar lines confluent, circumscribing second and third palmar areas. First palmar areas open upon the *outer* margin. All three palmar areas with patterns.

of reduction attained by the human or, at least, by the "Anglo-Saxon" palm.

Area.	No. of Patterns.		
	Right.	Left.	Total.
P_1	1	0	1
P_2	24	15	39
P_3	21	29	50
H	21	16	37
Th	2	2	4
Totals.....	69	62	131

In a single instance out of the hundred (Fig. 5) I have found a pattern upon each of the three palmar areas. As will be seen, this hand possesses also a well-marked hypothenar pattern, thus making it the

most atavistic one thus far noted. I am sorry not to be able to present for comparison a similar table of patterns in the sole of the foot, but I am delaying this in order to be able to include a like number of impressions. There is no doubt but that the total number of patterns occurring would be noticeably larger than in the above case.

e. Races and Sexes.—It would be of much interest to compare the sculpture of the palms and soles in the various races of men, as it is at least possible that *there may be sufficient difference to constitute important racial characteristics.* Although some efforts have been made to collect impressions of finger tips from several races, including some very primitive ones, there seems to have been, so far as I am able to learn, no definite attempt to make and collect impressions of entire palms and soles, a line of investigation which suggests many interesting results and which is of the highest importance in the farther development of this subject. Whether the question of sex need be considered in these investigations I do not know, but may state that practically all of the prints in my collection have been those of female subjects as are all of the prints published in this article with the exception of Figs. 3 and 4. The only race thus far investigated has been the so-called "Anglo-Saxon," that is, natives of the United States in great measure of English origin.

II. Palms and Soles in Identical Twins.

One of the most interesting portions of the present investigation has been the collection and study of the palm and sole prints of four sets of twins, three of which were of the type known as "identical," that is, of the same sex and otherwise so strikingly similar in facial expression and general appearance as to constantly mislead those with whom they are associated. On the ground of the well-known theory that such cases result from the development of a single egg, the blastomeres of which effect a total separation during the two-celled stage, while in the case of other twins there are two separate eggs, it is to be expected that the hand and foot prints of the first sort would be extremely similar, while those of the second would be no more alike than would be the case in any other brothers and sisters. An inspection of the figures here given (Figs. 10-21), which represent the complete prints of one set of identical twins and the hands of a second set, will show how remarkably the above supposition is verified. In fact, to one who has been in the habit of comparing such prints and who knows how great the individual difference is in the comparison of single hands or

feet the testimony of such a case as that shown in Figs. 10 and 11 alone seems a remarkable corroboration of the above theory, a point still more emphasized by the equal correspondence in the three other extremities, the left hands and the feet. Out of my entire collection of 100 palm-prints (not including the twins) it may be possible to find one or more cases where two hands bear a general correspondence in the disposition of the main lines and areas, but a comparison of the other hands and the feet of the two individuals would show the resemblance to be a chance one and limited to the single extremity. In the case of the three sets of identical twins, however, involving a comparison of eleven pairs of prints (one foot-print is missing in one set), each case, as seen by the six here given, shows almost the same degree of correspondence, thus removing all chance of coincidence. It seems to me, then, that we have in these comparisons *a definite and tangible proof*, far more reliable than that of general facial resemblance, *of the complete physical identity of such cases and one which can be accounted for only by the theory of origin from a single egg, the hereditary properties in which are equally and exactly divided by the first cleavage process.* To ascertain how these parts are related in the rare but actual cases of identical triplets and quadruplets would be of the greatest importance.

In one of the sets of twins I made also a careful comparison of the finger tips and found that nine out of the ten sets of patterns corresponded exactly, while in the tenth case, that of the right index finger, the two patterns were the exact symmetrical equivalent of one another, a case which might suggest to a Weismannian the somewhat fanciful idea of the mechanical reversal or other form of displacement of a determinant.

The completeness of identity in these cases is, however, not so great but that, both in the finger tips and on the surface of the palms and soles, *there are differences sufficiently marked to render impossible the mistaking of one print for another.* The identity is that of lines and areas rather than that of separate ridges, and the interruptions, branchings and other irregularities in these last, called by Galton the *minutiæ*, are entirely individual. Thus, notwithstanding the remarkable correspondences in the case of identical twins, which are naturally greater than could ever exist in any other two people, there are still enough individual differences to distinguish them from one another.

Concerning the fourth set of twins investigated, one in which there is no great similarity in general appearance, and which, in accordance with the above-mentioned theory, may be supposed to have been derived from two separate eggs, it may be sufficient to state that there is also

no correspondence in the prints of either palms or soles. For example, in the left hand of one of them, all the palmar areas are divided by the palmar lines, as in the case of Fig. 2, while in the left hand of the

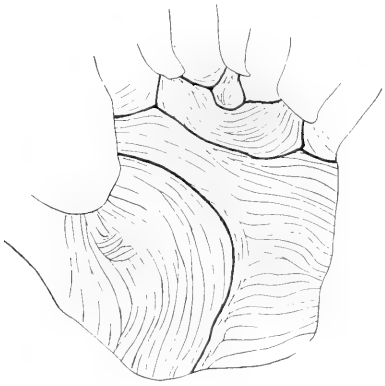

FIG. 10.

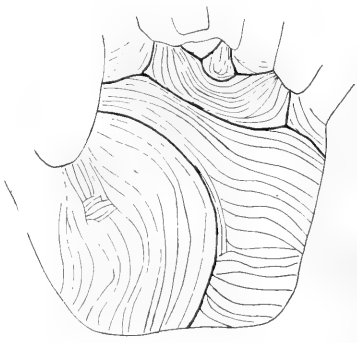

FIG. 11.

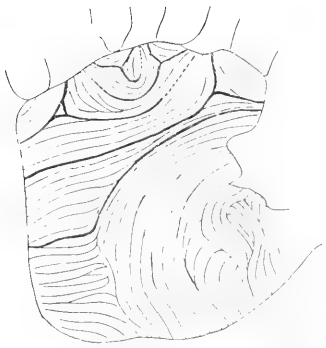

FIG. 12.

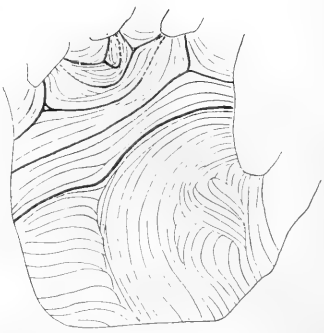

FIG. 13.

FIGS. 10 and 11.—Right hands of identical twins No. III. Tracing.*
FIGS. 12 and 13.—Left hands of identical twins No. III. Tracing.

*NOTE.—In this and the following figures of twins the even-numbered figures belong to one of the two individuals and the odd-numbered ones to the other.

LINES.	AREAS.	TRIRADII.
4 Palmar.....[$p_1 — p_2$]	3 Palmar.....[$P_1 — P_3$]	4 Palmar....[$tr_1 — tr_4$]
8 Digital { 4 outer [$do_1 — do_4$] / 4 inner [$di_1 — di_4$]	4 Digital .....[$D_1 — D_4$]	1 Carpal....[tr_1c]
1 Thenar.....[Th]	1 Thenar.....[Th]	
2 Carpal { outer..[co] / inner..[ci]	1 Hypothenar...[H]	
	1 Hypothenar addition[H^1]	
	1 Carpal.....[C]	

other the second palmar area is circumscribed, the first and third being partly confluent.

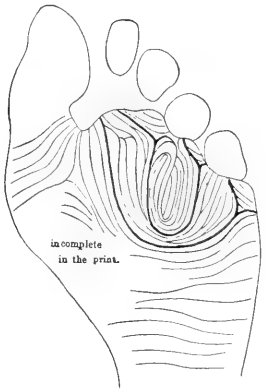

FIG. 14.

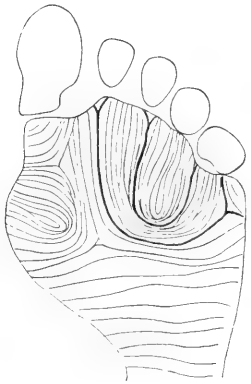

FIG. 15.

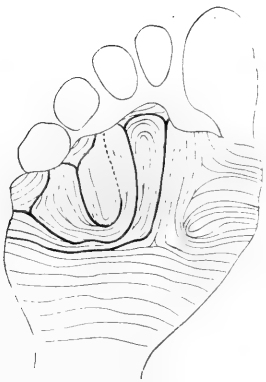

FIG. 16.

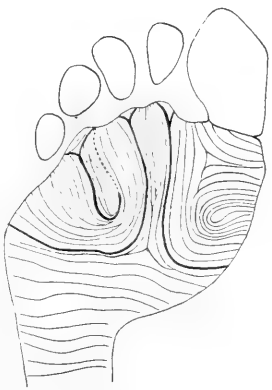

FIG. 17.

FIGS. 14 and 15.—Right feet of identical twins No. III. Tracing.
FIGS. 16 and 17.—Left feet of identical twins No. III. Tracing. The apparent difference is due to a slight change of position of a single triradius.

III. USE AS MEANS OF PERSONAL IDENTIFICATION.

(a) Palms and Soles vs. Finger-Tips.—Aside from the morphological and biological aspects of the subject as presented in the previous pages, there remains to be mentioned a side of great practical utility, a short discussion of which may not be out of place in this paper; namely, *the use of prints of palms and soles for purposes of personal identification.*

This is seen to be but a farther extension and application of the methods so carefully elaborated and so strongly urged by Galton through a long period of years, and to whom we are indebted for the establishment of the two points necessary for the success of such a system: (1) *the absolute permanence of these markings throughout life,* and (2) *the individual character of the markings and the impossibility of exact correspondence in any two individuals.* In Galton's system, however, the

Fig. 18.

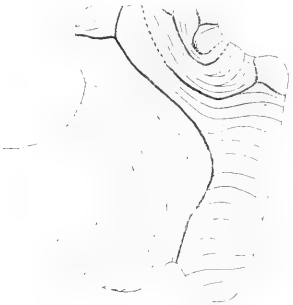

Fig. 19.

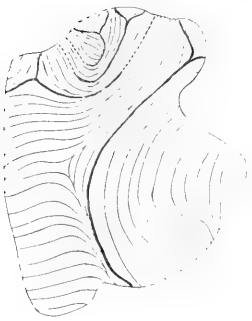

Fig. 20.

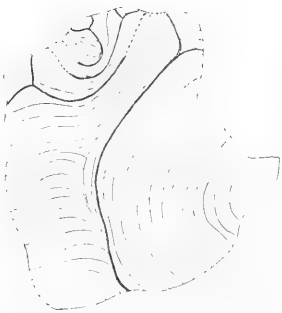

Fig. 21.

Figs. 18 and 19.—Right hands of identical twins No. I. Tracing.
Figs. 20 and 21.—Left hands of identical twins No. I. Tracing.

emphasis is laid upon the markings of the finger-tips, the area of which is so small and the patterns so similar that the work of identification rests upon the minutiæ, and demands too minute and careful a treatment to make it serviceable in the hands of an ordinary police captain or country sheriff.

Identification through palms and soles, on the other hand, deals merely with the topography of the main lines and areas as explained above, the plan of which, in an ordinarily good print, could be readily

ascertained without the use of a lens by a mere novice in the art, unaccustomed to the treatment of finer details. It may be granted that if the comparison were confined to a single member, cases might arise in which the study of lines and areas alone might lead to confusion between two different individuals, but the chance of such a coincidence extending itself to all four of the members is beyond the verge of possibility, especially when it is seen that even in the case of identical twins there are sufficient differences to avoid any excusable mistake.

(b) Comparison with Other Systems.—The main advantage of the palm and sole system over the one advocated by Galton is that it depends upon larger and more obvious details and thus becomes practical and applicable in cases where the latter could not be used. The ordinary system, based upon recognition of features and facial expression, is confessedly liable to much uncertainty, and the "Rogues-gallery" method of photography leaves much to be desired. Perhaps the most scientific system in practical use is that of M. Bertillon, which consists essentially of accurate measurements of the parts of the body, taken, so far as possible, of dimensions unaffected by fluctuations of bodily weight and degree of nutrition; but to subject a man to a thorough "Bertillonage," as the treatment is called, consumes considerable time, and requires the aid, both of expensive instruments and of an expert manipulator, advantages obtainable in the larger cities alone; besides which, the same process has to be undergone every time it is desired to compare a subject with a previously taken record.

Compared with all of the above the system here advocated possesses the following advantages:

1. The rapidity with which the records can be taken, the making of a set of prints requiring but about five minutes.

2. The inexpensive character of the necessary outfit, such outfit consisting essentially of a roller, a slate and tube of printer's ink.

3. The facts that prints may be taken anywhere and that a few minutes' instruction suffices to teach any man of common intelligence the methods of printing, interpreting and comparing the impressions.

4. The fact that the markings of the palms and soles are permanent and beyond the power of change, either voluntarily or involuntarily; a condition easily accomplished in general facial appearance, and not wholly guarded against by the Bertillon system.

5. The definiteness of the records, especially when marked by a conspicuous pattern, such as frequently occurs upon the hypothenar

area of the palm. Such a record is far more tangible and convincing than a correspondence in shape of nose or length of arm.

6. The rapidity with which a new set of impressions may be taken and compared with an old record, a single glance generally sufficing if in both cases compared the prints have first been interpreted and the lines marked with a colored pencil.

7. The ease with which a palmar or plantar condition may be expressed in words, enabling one to write or telegraph a formulated description, preparatory to the sending of the actual prints in case the correspondence is sufficient to warrant it. (As examples of such formulæ, see the captions given under some of the figures, quite incomplete but expressing the most prominent features.)

(c) Possible Applications of the System.—In the above paragraph the main attention was directed to the use of the system advocated for the identification of criminals, since the systems now in use deal mainly with that phase of the subject, but it plainly admits of a much wider application and might easily become inaugurated as a system of establishing identity in all cases, including cases of accident, of claimants for inheritances, of deserters from the army, and wherever such identification is necessary. Since the impressions in question are unchanged during life, a general law might require a set of impressions of all children born in a town or city to be filed away among the usual records to be accessible at all times whenever necessary for any adequate cause. Such records would best not be taken at birth, but at an age from perhaps five to ten years, at which time the impressions would be larger and more pronounced and the work of taking them would be much simpler. In the absence of a general law, it would be well for individual families to keep such records of their members, so that, in case of accident, identification would not have to depend upon the usual and often fallacious appearance furnished by the face.

Another line in which this system would be of great service would be in the official identification of Chinese, negroes, and other races, the features of which, at least to the Caucasian eye, offer hardly sufficient individuality to be at all times trustworthy. Should the government collect and catalog all the Chinese of the country, there would be no possibility of evasions of the Geary law, and the most of the expense assumed in establishing identity would be saved.

In closing, it would be well to recall to mind the celebrated case of the Tichborne claimant, which dragged through years of litigation involving an enormous expenditure, all of which would have

been saved and the case settled in five minutes had the Tichborne family made and kept the palm and sole records of the real Roger Tichborne.

Although the main object of this paper has been to express the matter in its morphological and biological significance, the practical appeal presented by the utilitarian side may excuse the digression of the last few paragraphs. *As a simple and infallible system for personal identification I feel that the claims of the one here described should be thoroughly presented and that the matter should be brought to the consideration of those in authority.*

Northampton, Mass., January 23, 1902.

THE PANCREATIC DUCTS IN THE DOG.

BY

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Associate in Anatomy, University of Chicago, from the Hull Laboratory of Anatomy.

WITH 14 TEXT FIGURES.

Of recent years it has been established that in nearly all mammals the pancreas is double in origin, one anlage arising from the duodenum, dorsal and isolated, the other ventral and connected with the bile-duct. Correspondingly there are two ducts which nearly always intercommunicate within that part of the gland which is formed by the union of the two anlages. That part of either duct which lies between this communication and the bowel wall may remain small, or disappear either wholly or more usually only in part. As a rule the part that disappears is that adjoining the bowel and only one pancreatic orifice is then present in the adult type. All these modifications of the embryonic condition may occur even within one species of animal, but one of them is most frequent, and is known as the "normal" for that species. To determine this normal and also the occurrence and frequency of other types, it is necessary to examine many individuals, more or fewer according to the inconstancy or constancy of the normal. The curves (Fig. 3) of relative frequency of the various types observed in the instances examined by the author show how fallacious may be inferences drawn from meagre early statistics.

The beginnings of the duct-radicles in the acini have been carefully studied by many observers since Langerhans's publication in 1869, the findings of the various workers being reviewed by Oppel, 1900. Magiarski has reconstructed the lobule of the pancreas. Much attention has also been given to the development of the pancreas, by which the variations of the ducts are explained. The following communication deals with the two ducts, their relation and distribution in the dog.

METHODS.—The topography was studied in dissections[1] and trans-

[1] 75cc. of formalin injected in to the A. carotis communis when the dog is killed will so harden the tissues in two hours that the form and relations of the viscera can be readily ascertained by ordinary dissection, one examination made in this way giving clearer, more exact and correct knowledge than many made without hardening. The

verse sections of dogs. The ducts were studied by dissection of fresh, of hardened and of macerated material; in sections; and chiefly by celloidin corrosion preparations. Casts of the duodenal parts (within the wall of the duodenum) of the bile and pancreatic ducts are made by ligating off the part of the bowel which receives the ducts, filling it and the bile-ducts with celloidin solution injected *via* the latter,

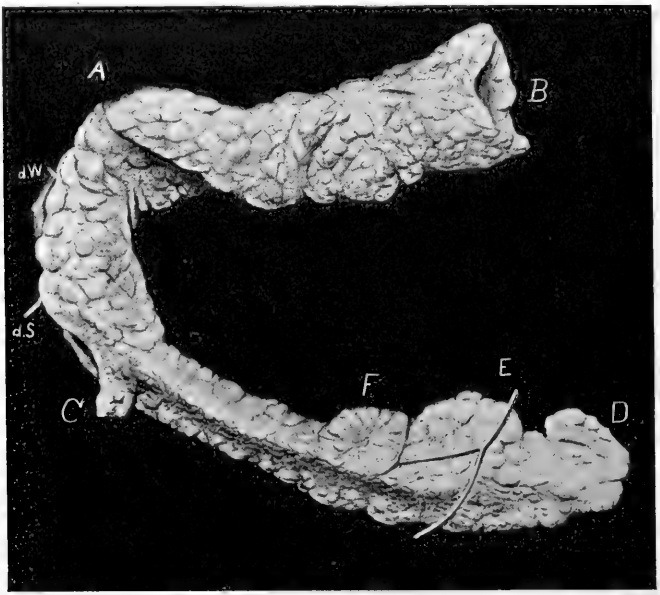

FIG. 1.—Pancreas of the dog with the parts brought into one plane. A B, omental or splenic portion, which lies nearly in a frontal plane and of which the expanded concave free end was applied to the left kidney; A C, epiduodenal or basal portion; C D, duodorsal portion. ACD, duodorsal division of Owen's description; this part lies nearly in a sagittal plane. E, branch from the arteria mesenterica superior crossing the left-hand surface of the pancreas on its way to the duodenum. F, lobe which is shown enlarged in Fig. 2.

then injecting the pancreatic ducts *via* the main duct within either the caput or the cauda pancreatis. These casts represent approximately

formalin may be diluted one-half with water and the last half of the solution may have starch or plaster of Paris and carmin added to it, giving a beautiful arterial injection. The abdominal viscera of the dog are so mobile that the animal should be placed in the ventral position before the injection, if the natural topography is to be studied. For the purposes of experimental operations done with the animal in the dorsal position, the topography of the organs in this position should be studied in material hardened with the animal on its back.

the lumina of the ducts within the bowel wall when the latter is stretched by distension. Casts of the lumina when the bowel is not distended are made by opening the first part of the duodenum along its ventral side to expose the orificial papillæ (vide infra), cleaning the mucous surface, dehydrating it with absolute alcohol and sealing up the orifices with celloidin, then injecting as above under low pressure. Or

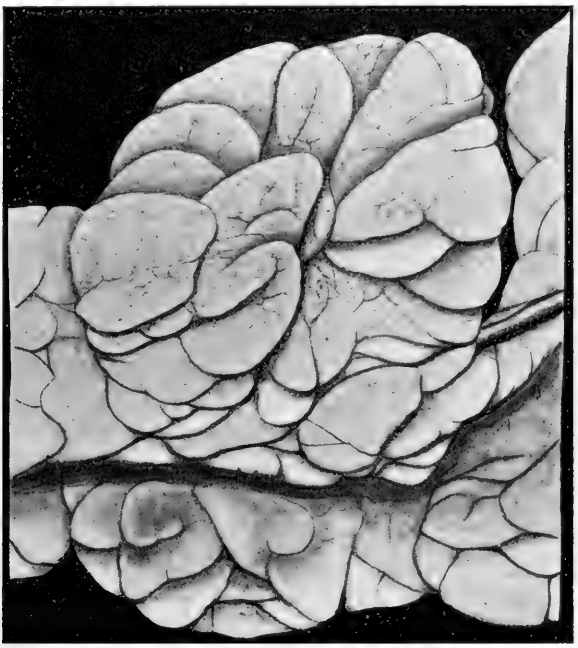

FIG. 2.—Enlarged view of lobe of pancreas of dog to show lobulation

the sealing up may be omitted and the relations of the bile-opening and Wirsung's can then be exactly determined as the injection solution and bile flow from the openings, pressure on the gall-bladder causing the bile to be expelled.

TOPOGRAPHY.—The pancreas in the dog is tongue-shaped, being 20-45 cm. long, 2-4 cm. wide and 1.5-2.5 cm. thick. Its size is quite variable, even in dogs of equal weight. It has three surfaces, of which two are broad and constant and one is narrow and may be discontinuous. The cross section is therefore like an isosceles triangle. The gland is somewhat loosely bound together by connective tissue into lobules and these again into larger lobules, until finally lobes of very

various sizes are formed. The free surfaces are nodulated and the margins are irregularly crenated. (See Figs. 1 and 2.)

The color is cream-pink to cream-red, varying according to the amount of venous engorgement in chloroformed animals. Usually several small lobes are much injected.

The gland is bent acutely on itself near its middle, giving it a ⋀ shape. The left limb, *cauda pancreatis* (termed *splenic* by Owen), is the shorter; it runs in the dorsal wall of the bursa omentalis, caudosinistralward from the pylorus, dorsal to the stomach, toward or to the left kidney upon which it may abut with a broad concave end; the a. and v. lienalis groove, its anterior border and the colon transversum

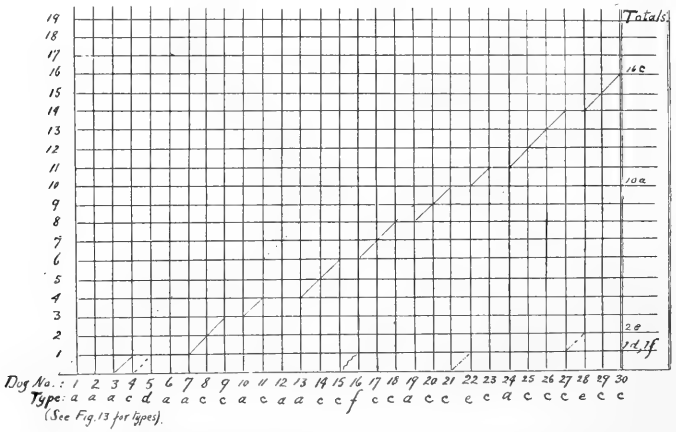

Fig. 3.—To show the frequency of occurrence of the types of the pancreatic ducts in the dog as observed in 30 consecutive cases.

is capped by its posterior surface. The right limb, *caput pancreatis* (termed *duodorsal* by Owen), extends in the mesoduodenum caudalward from the pylorus on the dorsal side of the duodenum nearly to the bend of the latter. It is longer, thinner and narrower than the left limb, and but for the influence of human anatomy, the terms "caput" and "cauda" pancreatis would here be reversely applied. The anterior 5-7 cm. rests on the duodenum, overlapping it somewhat on both sides; the remainder diverges dorsalward from the duodenum. The plane of this limb is sagittal while that of the left limb is principally frontal. At the apex of the ⋀ the pancreas is folded or twisted on itself so that the left limb is turned on its main axis through nearly 180°. The probable origin of this twist and the relation of the pancreas to the peritoneum are shown in Fig. 4, which represents diagrammatically the

primitive embryonic and the derived adult condition in the dog. The right limb is in the mesoduodenum and retains this position. The left limb is contained in the mesogastrium and the whole gland may be regarded as in one plane (A). The bursa omentalis being formed by a pocketing of the mesogastrium between the stomach and the pancreas, and the dorsal margin of the left limb of the latter being fixed,

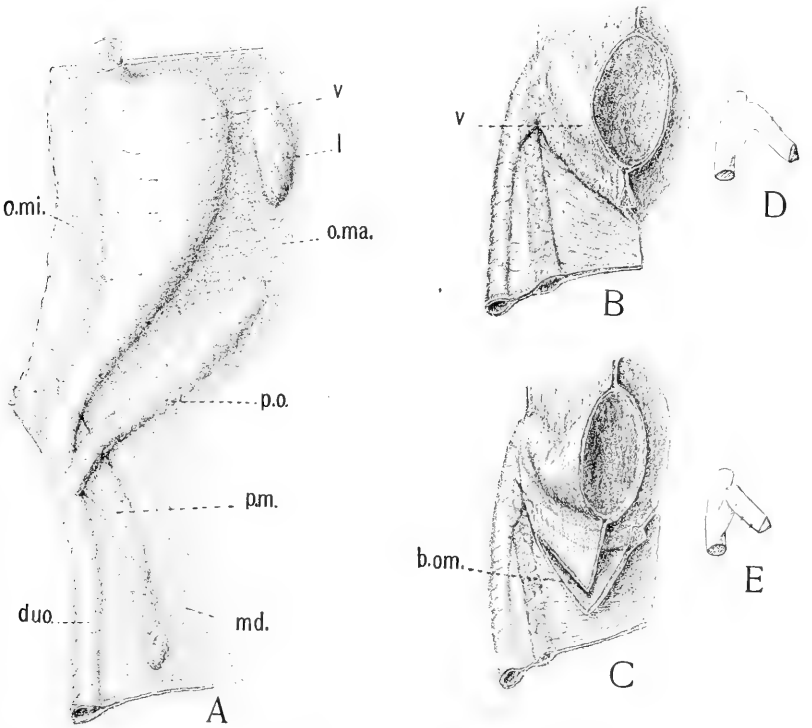

Fig. 4.—Scheme of development of the peritoneal relations of the pancreas in dog. In D and E are shown the part of the splenic division adjacent to the bowel. xx indicates the same border in each.

while the ventral margin is carried caudalward by the omentum majus, the twist results (C). The duodenal end is fixed or probably carried cranialward with the pylorus, while the splenic end is carried caudalward as the alimentary canal elongates and is thrown into curves. Thus the left limb becomes directed caudo-sinistralward. Except where it is in contact with the duodenum, the pancreas has everywhere a free surface covered by peritoneum.

Observations.—Pancreatic Orifices. Figs 5 and 6. There are two pancreatic orifices in the dog. One is in or close beside the terminal part of the ductus choledochus, this relation identifying it as the opening of the ductus Wirsungianus. It is about 0.3 mm. in diameter and is situated on the summit of a papilla in the mucosa of the dorsal wall of the duodenum, 3-5 cm. from the pylorus. It looks either into the ductus choledochus near the opening of the latter or caudalward on the free surface of the papilla, about 2 mm. from the bile-opening. In one instance celloidin injected into the bile-duct towards the bowel passed freely into the ductus Wirsungianus, having first closed the common orifice by precipitating in it.[2]

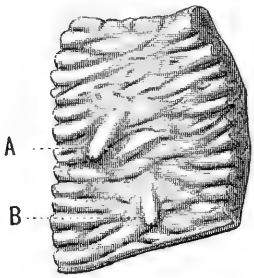

Fig. 5.

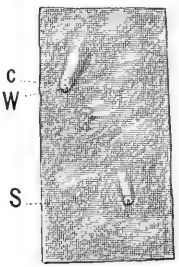

Fig. 6.

Fig. 5.—Part of dorsal wall of dog's duodenum, with orificial papillae, A, of ductus choledochus; B, of ductus Santorini. The bowel-wall was contracted and the mucosa much wrinkled.

Fig. 6.—Dorsal wall of dog's duodenum and orificial papillae. C, orifice of ductus choledochus; W, of ductus Wirsungianus; S, of ductus Santorini. The bowel-wall was relaxed.

The other pancreatic orifice is about 0.8 mm. in diameter, and is situated at the summit of a similar but smaller papilla 2-5 cm. caudosinistralward from the first. This is the opening of the ductus Santorini, distinguished by its isolation from the bile-duct.

These orificial papillae are 3-5 mm. high and are really the enlarged terminal part of ridges of the mucosa due to the duodenal parts of the ducts. Each ridge begins cranialward, culminates and terminates caudalward in the papilla. That nearer the pylorus is 7-10 mm. long and 2-3 mm. wide, and runs caudo-dextralward. The other is about 5 mm. long, 2 mm. wide and runs caudo-sinistralward.

The crests of the ridges and especially of the papillae are usually reddish owing to congestion of the blood-vessels there. The anterior papilla is often bilestained.

The real form of the papillae and ridges is seen only in sections of the duodenum. Fig. 7. As the bowel contracts, the ridges enlarge and numerous false rugae appear.

[2] This simulates injection of the pancreas by bile when the common orifice is obstructed by a gallstone.

Ducts (Figs. 8, 9, 10).—The ducts are conveniently described in three parts: (a) *intraglandular*, (b) *free*, between the gland and the bowel wall, and (c) *duodenal*, in the wall of the duodenum.

Ductus pancreaticus Figs. 8, 9, 10.—(a) Intraglandular part. The ducts arise in the lobules as the intercalated ducts into which the alveoli open. The interlobular ducts unite usually like a broad Y; one arm of the Y is usually short. The stems of the Y's in turn form arms of larger similar Y's. The planes of successive Y's tend to be at right angles. Occasionlly instead of a Y-form the ducts unite T-like and the arms of the T's may even be bent down a little. Each lobar duct originates by the union of the interlobular, and runs near the axis of the lobe toward the axis of the gland, being joined usually on all sides by various-sized lobular branches by which its caliber is continually augmented. In short broad lobes which do not extend far from the axial duct, the interlobular ducts unite Y-like nearly to the hilus of the lobe. Fig. 10. (The branching is dichotomous.)

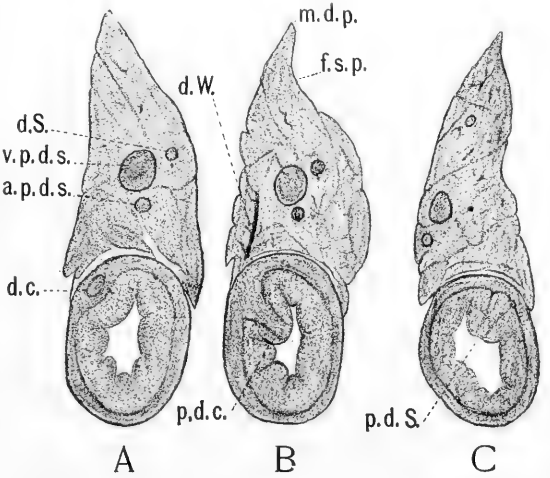

Fig. 7.—Sections of the duodenum and pancreas, to show the orificial papillae.

In or near the axis of each limb of the pancreas runs the main or axial duct. It arises in the free extremity of the limb by the union of the lobar ducts there. In its course towards the duodenum it receives lobar ducts on all sides, at wide angles of junction. The axial duct from the caput pancreatis meets and joins that from the cauda, near the duodenum, adjacent to the lower pancreatic orifice. Thence the main duct passes to the duodenum as:

(ii) Free part of ductus Santorini. This is most readily found on the left side of the duodenum. It is near the posterior part of that portion of the pancreas which is directly applied to the duodenum. The edge of the pancreas overlaps it and lobules of fat usually conceal its continuation into the bowel wall. Near it there is generally a large blood-vessel on the surface of the bowel. The duct can be located with-

out injury to the peritoneum by pushing back the edge of the pancreas, testing the fixity of the latter adjacent to the bowel, and looking for a whitish band sinking into the pale pink wall of the duodenum. This

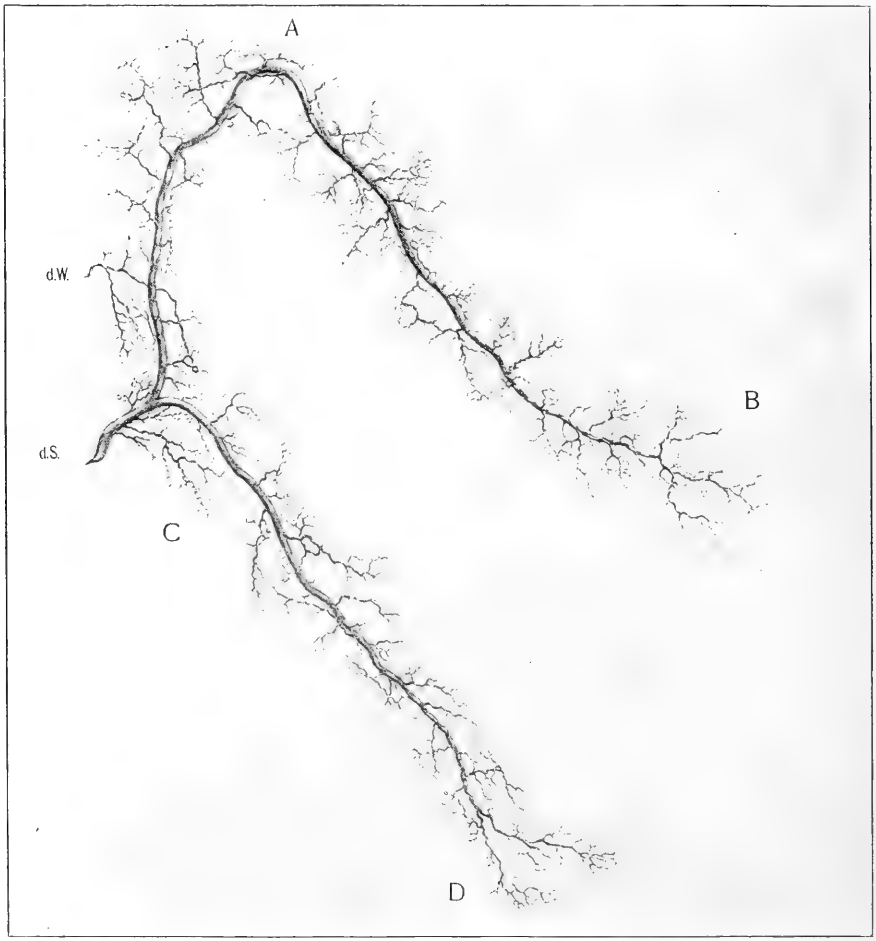

Fig. 8.—The pancreatic ducts in the dog. A B, omental portion; A C, epiduodenal; and C D, duodorsal portion.

part of the duct is directed somewhat caudalward, is about 2 mm. wide and 3-4 mm. long. It is flattened as it approaches the bowel. The union of the two axial ducts is within the gland as a rule, but may be at any point up to the surface of the duodenum.

(iii) Duodenal part of ductus Santorini (Fig. 12) is 5-7 mm. long,

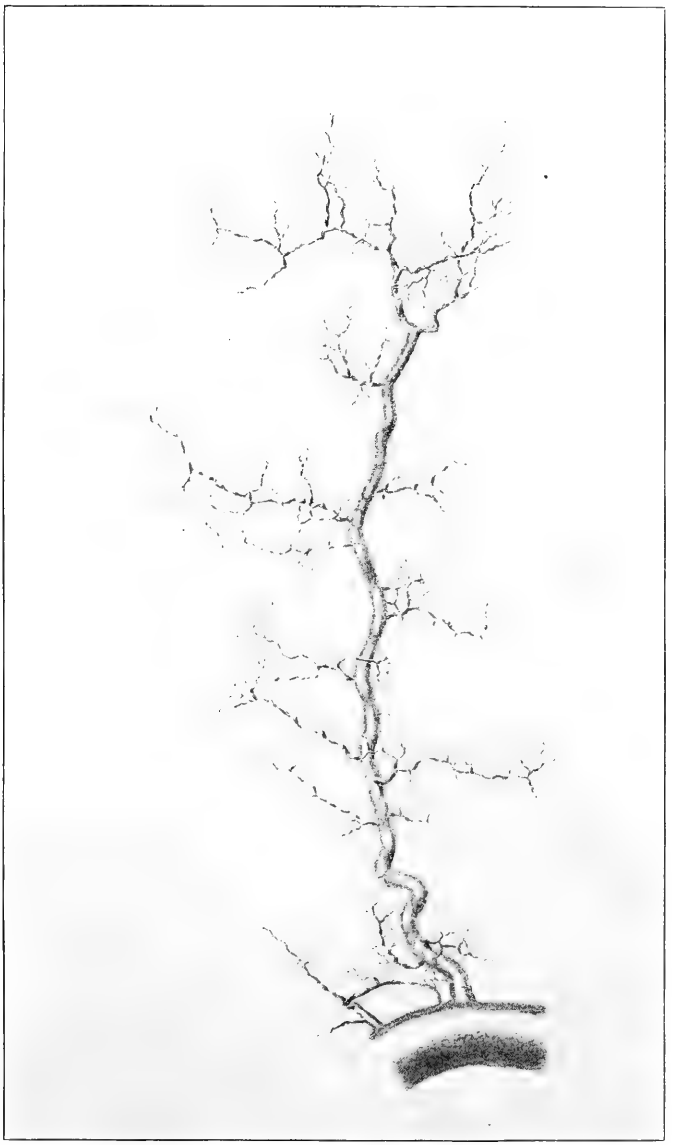

Fig. 9.—A lobular duct and its branches, from pancreas of dog.

2 mm. wide, and runs obliquely through the muscle-coats of the bowel, entering at an angle of 30°-60° with the longitudinal muscle fibres but curving so that this angle diminishes in the further course to the papilla.

Towards the base of the latter it is broadened in the plane of the muscle layers; the papillary part rises at an obtuse angle and tapers to the orifice.

Ductus pancreaticus accessorius (*Ductus Wirsungianus*).—(i) Intraglandular part lies in the right-hand part of the epiduodenal (*vide* summary) part of the pancreas, being directed cranio-dextralward, i. e. as if it came from the caput pancreatis. It is 1-5 cm. long and has as a rule the form of a side-channel from the main duct to the bowel (*vide infra,* Communications). It receives several branches, some medium-

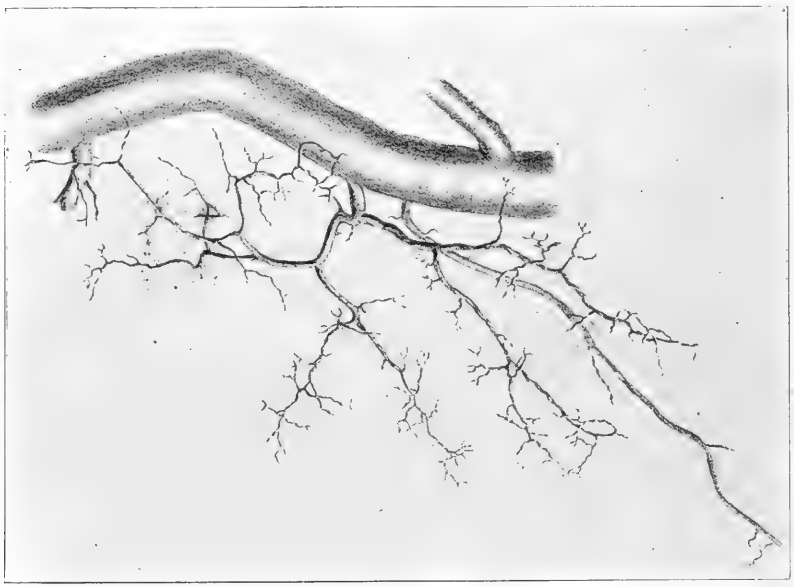

Fig. 10.—A lobar duct, showing dichotomous branching throughout. From pancreas of dog.

sized, but most are small. It is narrower in its middle part and wider towards its ends. It is like an interlobular duct and cannot be dissected out as clean and free as the axial ducts.

(ii) Free part of ductus Wirsungianus is to be sought on the right side of the duodenum. It is hidden under the pancreas and the best guide to it is the ductus choledochus. This passes under the pancreas for some distance before sinking into the duodenum, and is readily distinguished by its dusky orange-brown color, due to the bile in it and the vascularity of its walls. Its duodenal part shines through the bowel wall and can be traced nearly to its end, fading and finally disap-

pearing as it sinks deeper. A short distance caudalward and further under the pancreas from this point where the ductus choledochus disappears (1-2 cm. from the entrance of the latter into the bowel wall) the ductus Wirsungianus may be found. Its free part is 3-5 mm. long and usually 0.5-1 mm. wide. It may be known from a blood-vessel by its fixity, by its whiteness (or other color when artificially injected from the main duct) also by its sinking into the bowel wall and forming there a whitish area. It usually runs cranio-dextralward.

(iii) Duodenal part of ductus Wirsungianus (Fig. 11, D, E, F) is 3-5 mm. long, varies greatly in its direction, but generally runs cranio-dextralward at first, then curves so as to be parallel with the ductus choledochus. Its terminal part is very similar to that of the ductus Santorini (q. v.).

Casts of the lumen of the main duct often show numerous small knobbed projections from the surface. These can be best seen in injected sections. They are due to the small glands in the duct wall.

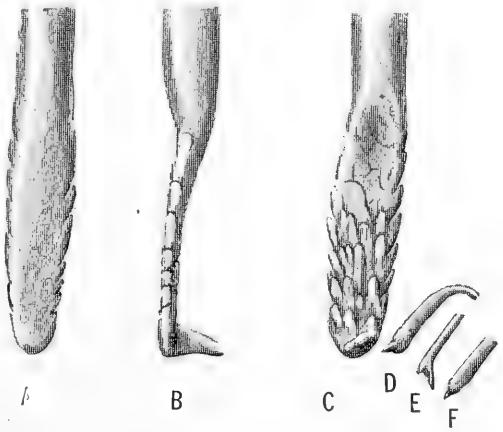

FIG. 11.—Celloidin casts of the duodenal part of the ductus choledochus (A, B and C) and of the ductus Wirsungianus (D, E and F), made with the duodenum distended. C and D, ventral aspect; B and E, seen from the left; A and F, dorsal aspect. The scale-like appearance in A, B and C is due to the crypts.

It will be seen (Figs. 1, 2, 8, 9, 10) that there is necessarily a close correspondence between the form and size of the ducts and the shape and lobulation of the gland. The gland and the main ducts are long: the gland is narrow and the largest duct branches are relatively short; the lobes and lobules are very various in size, as are also the duct branches.

It may also be observed (Figs 5 and 6) that the free and duodenal parts of the ducts run outward from under the pancreas. Or stating this fact from the standpoint of development, and considering the ducts to run *from* the bowel, the two ducts are directed toward each other as they go to the pancreas, this direction giving a reminiscence of the approaching, crossing and fusing of the two original parts of the gland.

C. Communications between the two pancreatic ducts.—In no instance of about 40 determinations was either duct absent. The relation between the ducts was exactly determined in thirty cases, the findings being reducible to five types, diagrammatically represented in Fig. 13; A and B belong to one type but differ in that in A the ductus Wirsungianus has a longer and more tortuous course and joins the main duct nearer its bifurcation. A is more frequent than B. This type (A or B) occurred sixteen times in the thirty; type C, ten times; D, once; E, twice; and F, once. When the preliminary examination of the free part of the ductus shows that the ductus Wirsungianus is large, type E or F is very probably present, and should be carefully examined for.

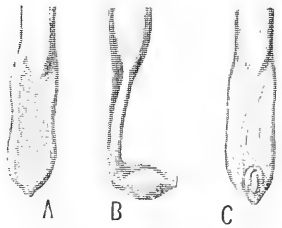

Fig. 12.—Cast of the duodenal part of the ductus Santorini, A, seen from the dorsal side; B, from the right; C, from the ventral side. The duodenal wall was stretched by distension of the bowel.

In the instance in which the two ducts were not in communication, the pancreas was readily separable into two parts, one passing from near

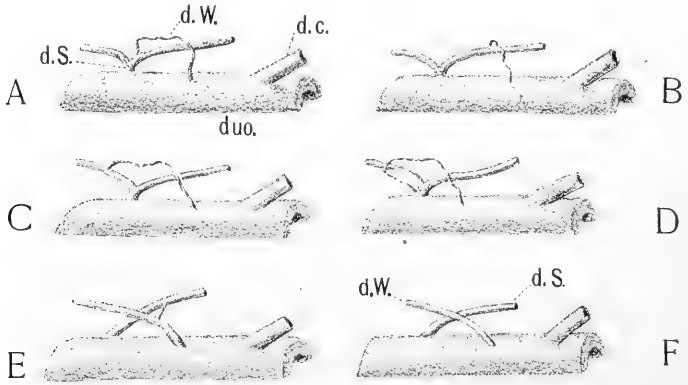

Fig. 13.—The terminal parts of the bile and the pancreatic ducts, and the dorsal wall of the duodenum. From dog. Diagrammatic. In A and B the ductus Wirsungianus joins the main duct from the cauda pancreatis; in C, it joins the duct from the caput pancreatis; and in D it joins the main duct. In E and F the two pancreatic ducts are about equal in size. In F, they do not communicate and each is confined to its embryonal field.

the pylorus caudalward (caput pancreatis), containing the ductus Wirsungianus, while the other extended cranialward along the duodenum from the lower pancreatic orifice to the pylorus and then bending to the

left formed the left (splenic or omental) limb (cauda pancreatis); it contained the ductus Santorini, which did not bifurcate. The pancreatico-duodenal vessels ran between the two divisions of the pancreas. There were in fact two pancreatic glands present, (caput pancreatis) right, the other (cauda pancreatis) to the left. In all the other instances, in which the ducts were united, the loop formed by the ducts from one orifice to the other arched over the pancreatico-duodenal vessels (Fig. 14).

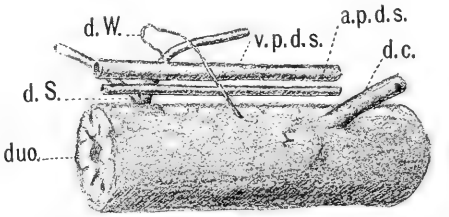

FIG. 14.—Part of the duodenum, ductus choledochus, pancreatic ducts and superior pancreatico-duodenal vessels, seen from the right side.

Types not met with but which are possible are:

(a) Ductus Wirsungianus the larger.

(b) Either duct absent.

(c) Only one pancreatic orifice.

The varying size of the ductus Wirsungianus indicates that all these types will be met with in a sufficiently large series.

REMARKS.—1. A consideration of the types (Fig. 13) of the ducts in the adult leads to the following conclusions regarding the pancreas of the dog:

(a) The pancreas has a double origin, arising from at least two anlages, which are placed on opposite sides of the pancreatico-duodenal vessels, but unite beyond these so as to form an arch over them. (Fig. 14).

(b) The original fields of the two ducts (and of the two anlages) are: (i) ductus Wirsungianus (ventral anlage), the caput pancreatis (duo-dorsal segment) and the right half of the basal or epiduodenal (*vide infra*) segment; (ii) ductus Santorini (dorsal anlage), the remainder of the gland.[3] By the anastomosis which occurs where these two fields are contiguous, the ductus Santorini takes over the drainage of the field of the ductus Wirsungianus beyond the anastomosis.

(c) These facts justify giving distinctive names to the two segments of the right limb of the pancreas: the anterior part, applied to the duodenum consists of the proximal or basal part of the product of each

[3] This accounts for Flexner's observations in his study of experimental pancreatitis. He injected the ductus Santorini—not the ductus Wirsungianus—with various fluids, and observed immediate and subsequent effects most marked in the *splenic* and *basal* parts of the gland.

anlage, and may be termed *basal* (portio basalis); the part that diverges from the duodenum may be designated *duodorsal* (portio duodorsalis) (making a restricted application of Owen's term).

The left limb of the pancreas, from its position, may be termed omental (portio omentalis).

2. The names "ductus pancreaticus" and "ductus pancreaticus accessorius" do not always coincide with "ductus Wirsungianus" and "ductus Santorini." The latter are applied with reference to relation with the ductus choledochus, which is constant. The former are applied with reference to relative size of the pancreatic ducts, which varies. For the purposes of human anatomy, of comparative anatomy, and of embryology, it is desirable to have exact names. The modification which the ducts and parts of the pancreas undergo seems to necessitate the retention of two pairs of terms, one pair based on organogeny, the other on the morphology of the adult gland. Instead of the indefinite and unscientific eponymic terms "Wirsungianus" and "Santorini" there may be used:

(1). *Ductus hepatopancreatis* seu *ventropancreatis*—the duct arising only from the ventral or hepatopancreatic anlage.

(2). *Ductus dorsopancreatis*—the duct derived only from the dorsal pancreatic anlage.

The *ductus pancreaticus* is the main duct (in the adult gland) whether simple or compound in origin. The *ductus pancreaticus accessorius* is the smaller or subsidiary duct, or residue of a duct, when two are present in the adult gland.

In conclusion, it is a pleasure to thank Professor L. F. Barker and Professor J. M. Flint for many kind suggestions throughout the course of this work.

ABBREVIATIONS USED IN THE FIGURES.

a. p. d. s., arteria pancreatico-duodenalis superior.
v. p. d. s., vena pancreatico-duodenalis superior.
b. om., bursa omentalis.
duo., duodenum.
d. c., ductus choledochus.
d. S., ductus Santorini.
d. W., ductus Wirsungianus.
f. s. p., facies pancreatis sinistra.
m. d. p., margo pancreatis dorsalis
md., mesoduodenum.
o. ma., omentum majus.
o. mi., omentum minus.

p. m., pancreas from dorsal anlage.
p. o., pancreas from ventral anlage.
p. d. c., papilla of ductus choledochus.
p. d. S., papilla of ductus Santorini.
l., lien.
v., ventriculus.

AUTHORS CONSULTED.

1. Ellenberger und Baum. Anatomie des Hundes, Berlin, S. 312-313, 1891.

2. Flexner, S. Experimental Pancreatitis, J. H. Hosp. Rpts., Baltimore, Vol. IX, p. 743-771, 1900.

3. Helly, K. Zur Pankreasentwickelung der Säugethiere. Arch. f. mikr. Anat., Bonn, Bd. 57, S. 271-335, 1901.

4. ——— Bemerkungen zum Aufsatz Völkers: Beiträge zur Entwickelung des Pankreas bei den Amnioten. Arch f. mikr. Anat., Bonn, Bd. 60, Hft. 1, S. 174-176, 1901.

5. Leche, W. in Bronn's Klassen und Ordnungen des Thierreichs. Bd. VI, Abt. 5, S. 1108-1109.

6. Leisering. Anatomie der Haussäugethiere, by Leisering-Mueller-Ellenberger, 7 Aufl., S. 448-450, 1890.

7. Opie, E. L. Experimental Disseminated Fat-Necrosis, J. H. Hosp. Repts., Baltimore, Vol. IX, p. 859-876, 1900.

8. Oppel, A. Lehrbuch der vergleichenden mikroskopischen Anatomie, Jena, 1900, Bd. 3, S. 742-870. Contains a review of the literature on the pancreas up to 1900, and gives an extensive bibliography.

9. Völker. Beiträge zur Entwickelung des Pankreas bei den Amnioten, Arch. f. mikr. Anat., Bonn, Bd. 59, Hft. 1, S. 62-93, 1901.

THE MORPHOLOGY AND DEVELOPMENT OF INTESTINAL FOLDS AND VILLI IN VERTEBRATES.[1]

BY

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From the Laboratory of Histology and Embryology, Cornell University.

WITH 2 TABLES AND 87 FIGURES.

For many years confused ideas have been prevalent concerning the form and occurrence of mucosal folds and villi in the different divisions of vertebrates, and even yet it is not entirely clear just where folds cease and villi begin. There are great variations in occurrence even in closely related forms, but much of this confusion may be due to the various methods of study which have been employed. Comparatively few investigators have made an extended study of the folds and villi in the different classes, although quite a number have made careful investigations of a single vertebrate class, and much work has been done upon the folds and villi of different species when the general digestive system of a typical form was described.

The phylogenetic study of an organ often reveals many perplexing structures which may render conclusions very doubtful. In such a study of folds and villi, difficulties present themselves which are almost insurmountable without the aid of embryology. So in order to obtain a correct idea of the primitive as well as the more advanced forms of mucosal elevations, it is necessary not only to make rather extended observations of numerous species of all great groups, but also to trace the development in several types of vertebrate embryos. The possibilities for work along these lines are very great, and in this investigation, only so far as time and material would allow, such work was attempted. An effort was made to determine as clearly as possible the limits of

[1] This paper was presented to the faculty of Cornell University as a thesis for the degree of Doctor of Philosophy, May 1902. I wish to express my grateful appreciation of the facilities placed at my disposal by the Department of Histology and Embryology, and for the aid and encouragement of the instructing staff. I am also greatly indebted to Prof. B. G. Wilder of the Department of Vertebrate Zoology, who very kindly enabled me to examine many valuable specimens which are in the possession of that Department.

folds and villi; to describe the character of folds and villi in some forms not already described as well as to supplement what was known about others; to determine, as far as possible, the form of folds and villi characteristic of different groups of vertebrates; to throw some light upon the development of villi in different forms—first, by the general survey of conditions existing in adult species; second, by embryological study of a few types.

Methods.

In order to obtain folds and villi in their usual form, fixation must be used which will not cause the muscular fibers of the *muscularis mucosæ* to contract and so produce unusual shapes. The intestines were usually injected and placed in the hardening fluids before the tissues had ceased to be irritable, so almost any of the fixing fluids which are rapid in their action were not desirable. Although formalin acts quickly, it was found to be very satisfactory, as the villi appear only slightly distorted, but it was not so useful when the finer structural elements were to be preserved. Müller's fluid, or a solution of 3 per cent dichromate of potash, does very well for preserving villi, in that the action of these fluids is slow and villi are seldom contracted; but when these fluids are employed, the epithelium is usually exfoliated. At times a rather high percentage of alcohol was efficient as a preservative, as the action was not rapid.

After hardening the intestines either in the fixing fluid or in alcohol, rather thick sections were cut in collodion; the thickness of the sections depending upon the size of the villi to be examined. Examinations of the whole intestine under liquids often helped to determine the character of folds or villi. When the muscular coats were thin enough, bits of intestine were stained with hydrochloric acid carmine, and mounted mucosa up. When this was not possible, villi were examined which had been isolated by scraping or cutting from the mucosa.

For counting and measuring folds and villi, various methods were used. The most satisfactory, but not always applicable, method was by means of an ocular micrometer in squares. When villi were very numerous and the walls of the intestine thick, they were counted by removing a measured bit of mucous membrane and isolating the villi from it, counting their tips or bases until by repeated trials, uniform results were obtained. In every case the intestines were studied by as many different methods as possible and the results compared.

The following is a list of the methods ordinarily used for each specimen:

1. Fixation by two or more methods.

2. Naked eye examination, with isolation of villi to be studied under a lens or microscope.

3. Cutting free-hand sections if possible.

4. Mounting mucosa up after staining, if the muscular walls were thin enough.

5. Cutting serial sections from tissues imbedded in paraffine or collodion.

For the work on embryological development of folds and villi the usual fixing and staining fluids were employed.

Character and Occurrence of Folds and Villi.

Mucosal elevations of the intestines may be considered under four heads: first, such as the typhlosole and spiral valve; second, valvulæ conniventes; third, simple more or less longitudinal mucosal folds; fourth, villi.

The first and second divisions hardly come under the scope of this discussion, but will be briefly considered.

It may be said that the typhlosole of lamprey is a simple or partially developed spiral valve. In Elasmobranchs, a high development of this organ is reached; here the spiral valves vary much in complexity, but are traceable directly or indirectly, with the help of embryology, to a simple type such as the typhlosole of lamprey. The Ganoidea apparently have more or less distinct remnants of spiral valves, while this peculiar type of intestinal folds is found to have reached a high development in the Dipnoi and Holocephali as well as the Elasmobranchii.

Ruckert, 96, described the development of the spiral valve of Pristiurus from spiral foldings of the mucosa; but it seems to me that the spiral valve is like a folding of the whole thickness of the intestine rather than of the mucosa and submucosa alone, and in the course of time the infoldings may have lost much of the original character of the infolded intestinal wall. I shall not attempt to explain just how or when this infolding took place, but it seems probable that it began before or with the formation of a simple spiral fold like the typhlosole. This infolding may be considered as similar to several coils of intestine which have come into very close contact; no essentially new structure is formed, simply a modified intestinal tube. The folding of the spiral is probably as much for retarding the progress of the food as for increased absorption area, because the absorbing area is not much greater than that of a coiled, small caliber intestinal tract; for where

the spiral valve is present, the intestine, although of considerable diameter, is very short. Hence, a very important difference, physiologically, may be noticed between the spiral valve and valvulæ conniventes, the first developed especially for retention or retardation of the food and greater compactness, and the second, formed especially for increased absorption area. The important difference already hinted at, is that spiral valves were formed from an infolding to a greater or less extent of the whole muscular walls as well as of the mucosa and submucosa, while valvulæ conniventes were formed by a simple fold of the mucosa and submucosa. This theory is partially based upon the following facts.

In the spiral valve of Lepidosteus and Amia, there is more muscular tissue than could have been derived from a double layer of the muscularis mucosæ.

There is a spiral fold in the cæcum of rabbits. When rabbits are born, this structure is not fully developed, its presence is marked on the exterior by constrictions, sections across these constrictions show the developing folds on the ental surface and the muscle of the muscular coats can be traced for some distance into the spiral core (Fig. 41). Of course this fold in the rabbit may not be homologous with the spiral valve of other forms, and the hypothesis outlined above requires much further investigation before it can be substantiated.

It seems that man is the only animal who can justly lay claim to *valvulæ conniventes*. It might naturally be supposed that some of the anthropoid apes would possess structures corresponding to some stage of these folds, however, careful examinations of specimens and a review of the literature on apes did not bring to light any clue to such structures.

The *valvulæ conniventes* of man begin their development shortly before birth and do not reach their full height until long after the villi are formed. They begin as small, transverse, semi-ringlike thickenings in the submucosa, these grow more prominent, those below the pylorus some distance become prominent more rapidly than others; as these thickenings project inward, they carry on their surfaces the villi of the mucosa (Fig. 42). The structure and arrangement of valvulæ conniventes in the adult are so well known as to require no redescription.

One particular more than another which may be worthy of note is the following: Brooks, **92**, and Kazzander, **92**, described valvulæ conniventes and both recognized in some cases, a spiral arrangement of the transverse foldings. Just what this signifies, it is difficult to

decide, but, to me, it seems doubtful that it indicates either a progress towards or a remnant of, a perfect spiral valve. If it indicates a developing spiral valve, it would probably develop in a different manner, as those of Elasmobranchs do. If a remnant of a once perfect spiral, why do we not see more forms between man and fish having this peculiar folding?

Simple, primary[2] folds of the mucosa are easily distinguished from secondary folds, such as the spiral valve and valvulæ conniventes. The simple folds are usually found when no villi are present, while the spiral valve or valvulæ conniventes may have folds or villi upon them. Then, too, the simple folds are more or less longitudinal in their distribution. They vary from thin or thick, slightly wavy to very wavy or zigzag forms, and sometimes they may have a net-like arrangement where some parts of the folds extend transversely. In no case do transverse folds entirely replace the longitudinal ones. It might seem from this that the original direction of the folds was longitudinal, but in the course of development, parts of the folds in some cases had come to take a transverse direction.

A villus is a projection of the intestinal mucous membrane covered with columnar epithelium and having its core made up of adenoid connective tissue; it also has a network of capillaries in this core supplied with one or more arterioles. In the central part of the villus and surrounded by the blood vessels, are one or more lacteals. Usually a villus has its base diameter much less than its height, but there are some exceptions to this. The distinction between a very broad villus and a small fold cannot be a sharp one; usually, folds are comparatively few and continuous, while villi are distinct and very numerous.

Villi are of many forms; they vary from thin plate-like kinds with square or rounded edges much like parts of folds, to long or short, slender or thick, cylindrical forms with rounded or sharp tips.

For convenience, the occurrence of folds and villi in different animals will be spoken of together. As other elevations of the mucosa have been discussed as far as it is necessary for the purpose of this paper they will simply be referred to occasionally. In many groups the conditions existing in different species have been carefully described by many investigators; besides giving a brief review of a few more important of the many forms studied by these, there will be added occasional supplementary descriptions and descriptions of forms hitherto undescribed.

[2] It seems best to consider as primary folds, those which are the simplest and the first to develop. The secondary folds develop later and independently.

Besides the typhlosole of Petromyzon, simple longitudinal folds are found in Cyclostomata; and the intestine of Myxine, according to J. Müller, 45, is entirely smooth except for a few very small longitudinal folds.

Villi have been described upon the spiral valves of Selachians by Pillet, 85. My own observations seemed to confirm this description.

In the intestines of Ganoids which I have been able to examine and from descriptions by various investigators it seems evident that net-like or zigzag, closely placed folds occur, but in Amia, the net-like fold arrangement is in many places broken up into free projections which are true villi (Hilton, 1900).

Many varieties of mucosal elevations occur in different forms of Teleosts. Villi have been described in *Orthagoriscus mola* by Rudolphi, 28, Owen, 68; *Esox lucius*, Grimm, 66. Meckel, 29, and Ratke, 37, have also described villi in a number of species. In the rather limited number of Teleosts I have been able to examine, no true villi were found, and in *Esox lucius* in which villi have been described, a simple network of true folds was found which might be taken for villi in sections, but could not otherwise be mistaken for them.

Three forms of folds were found in Teleosts:

1. The rather closely placed more or less wavy, thick folds; such as Macallum, 84, describes for *Amiurus catus* and those found in *Amiurus nebulosus* and *Perca flavescens* (Fig. 7).

2. Very low zigzag folds which are scarcely more prominent than those on the finger tips; such as those found in *Catostomus catostomus* one of the Catostomidæ (Figs. 5 and 10), or those of *Notropus cornutus* and *Phimephales notatus* of the Cyprinidæ.

3. Net arranged folds as those of *Esox lucius*.

There seems to be very little literature upon the mucosal elevations of Dipnoi and what little there is deals almost entirely with the spiral valve.

Leveschin, 70, described villi in *Salamandra maculata*, but in all American tailed Amphibia no villi were found. About the simplest condition was found in *Siren lacertina*, where the intestine in the specimens examined was smooth. The most common form of fold was a simple longitudinal rather straight or slightly wavy kind which in many cases was rather thick; such folds as are found in Necturus, Gyrinophilus, Amblystoma, etc. (Figs. 3 and 4). Another form of fold was found in the intestine of Amphiuma. In this animal the folds are quite numerous and zigzag with rather sharp angles (Fig. 2). Villi have been described in very few Anura. The folds described in Euro-

pean species and found in American forms are either somewhat net-formed, or much like those of Necturus, Amblystoma, etc., but may be larger and slightly more wavy.

Villi have been described by Langer, **86**, in the genus Bufo; these villi are said to be long and overlap each other; but in *Bufo agua* Klein, **50**, described net-formed folds. In *Bufo lentiginosus americanus*, I found a net arrangement of folds for a short distance beyond the pyloric valve. Isolated parts of these folds approximate villi (Fig. 9).

There are a few descriptions of villi in reptiles. Meckel, **17** and **19**, has described villi in quite a number of forms and folds in some. Stannius, **46** and **56**, described villi in crocodiles and in chamæleon. In the American representatives of this class which were available for study, no villi were seen and in the forms studied four different types of folds were found:

1. Long, plate-like, regular, parallel folds, which are rather thin, but usually quite high and thickly placed. Such folds are found in several species of turtles (Fig. 1).

2. Parallel wavy folds, similar to those of Necturus, etc., such as are found in some of the Colubridæ and Crotalidæ and some of the Lacertilia.

3. Very zigzag folds such as are found in *Bascon constrictor* (Fig. 8). In this species, the common blacksnake, besides these very zigzag folds, areas were found where the intestine either artificially or naturally, is of small diameter and at these places the zigzag folds are replaced by parallel flat longitudinal plates or folds.

4. Low, very zigzag elevations which differ from those of Cyprinidæ in being more prominent. In *Alligator mississippiensis* (Fig. 6) this type of fold was found.

The mucosal folds and villi of birds have received much attention from Owen, **67**, Gadow, **69-79**, Rudolphi, **80**, and a number of others. The second of these authors quoted has done much more than any of the others and the following types of structures found in American forms correspond very closely to the structures given by Gadow for European species.

There are three of these divisions in which an attempt is made to show the occurrence of folds and villi in birds.

1. Folds and no villi.

(a) Zigzag folds, such as are found in some of the sparrows and described by Gadow in Monticola, Euphone, etc.

(b) Net-arranged folds, as in Murre (*Uria troile*); and in the genus Sturnus of Passeres described by Gadow.

2. Both folds and villi in the same intestine.

(a) With the folds net-formed. Gadow describes such a condition in the Allidæ. I was unable to find such a condition in any forms examined.

(b) With zigzag folds and usually their plate-like villi regularly arranged. Under this head come the mucosal elevations of *Nycticorax nycticorax,* night heron, Scoter duck or *Oidemia deglandi, Lars argentatus smithsonensis,* and a number of others. Gadow has described this condition for Oriolus, Lanius, etc.

3. Villi and no folds.

(a) Some of the villi in parallel rows, or otherwise regularly arranged. In Flamingo, Owen, 69, described short villi in long parallel zigzag rows. Similar conditions are described by Gadow, 49, in Scolapax, Limosa and others. In a specimen of domestic duck some conditions were noticed which differ in several respects from any other form studied and so will be given in more detail.

Domestic duck.—In the upper part of the intestine of duck, the villi are quite regular and numerous. They are thin at first, square for the first part of their length, but near the tips, triangular shaped with sharp points. In many cases, two villi fit together; on one side the outer edge of one is thickened and each thick edge laps upon the thin edge of the other (Fig. 43). The villi are in quite close contact throughout their whole extent. This pointed sort of villus extends down the intestine for about 82 cm. and they are about 1.2 mm. high by .3 mm. broad at the base. There are about 12 villi to the sq. mm. For the last 63 cm. of the small intestine the villi become quite uniformly .6 mm. high by .3 mm. broad. They are thin, oblong plates with even edges and right angles. These villi are arranged very regularly and there are about 15 to the sq. mm. Upon looking down upon the surface of the intestine, it is seen that each side of the top of each one has presented to it the edge of the top of another villus (Fig. 44). In the cæca, the villi are somewhat smaller and elongated, .4 mm. by .15 mm. broad at the base (Fig. 45). In the large intestine, the villi are thin plates that have straight edges with right angles and are screen shaped. Each top looks like a broadened "v" when looking directly down upon it. These villi are about .6 mm. high by .8 mm. broad and are arranged in regular, parallel rows, the convexity of one villus fitting into the indentation of the other (Fig. 46). There are four or five of these to the sq. mm. In each cæcum there are about 7500 villi; in the large intestine about 16,000; in the small intestine, 440,000, or nearly 500,000 in all.

(b) With villi which are not regularly arranged. This condition exists in the intestine of ruffled grouse, or *Bonasa umbellus,* domestic turkey, chicken, humming bird (*Trochilus colubris*), red-eyed vireo (*Vireo olivaceus*) and many others. It has been described by Gadow for a number of forms including the genus Corvus of Passeres.

In the following table (page 468) particular attention is paid to the form, size and number of villi in several species of birds which were carefully studied.

It may be seen from the preceding descriptions and the table on next page that there are really only two distinct forms of villi with many slight variations. These are the thin leaf-like form and the tooth-shaped columnar form. It may also be observed that in birds villi or folds are found to a greater or less extent in the large intestine and cæca as well as in the small intestine.

The occurrence of folds in the intestines of adult mammals seems to be exceptional and villi are in most cases entirely confined to the small intestine.[3]

Cuvier, 10, Meckel, 26, Owen, 47, Leydig, 47, and others, have described ring-like somewhat spiral or oblique folds for Ornithorhynchus; these are most numerous near the pylorus and extend into the first half of the colon, leaving the caudal half smooth. Small "secondary" projections were described on these folds by Oppel, 97, but he was unable to state definitely the homology of either of these structures.

Folds have also been described in some of the Edentata, Cetacea and Sirenia, by Meckel, 19, Rapp, 37 and 43, Eschricht, 49, and several others; Leydig, 57, Forbes, 79, have described large folds in elephants.

Villi are usually described upon the folds in elephants. In a number of species of mammals where folds have been described no reference is made to the occurrence of villi. Unfortunately I was unable to examine any specimens of the mammals in which these folds have been described.

The forms of villi in mammals may be grouped under four heads:

1. Thin, leaf-like or plate-like villi.

(a) With square corners, as found in the intestine of musk-rat (Figs. 14-16).

(b) With rounded edges, as in the duodenum of man, the intestine of apes and monkeys and a number of rodents (Figs. 31-23, 25-27, and 34, 35, 37).

[3] Little mound-like elevations are present in the cæcum of rabbit. These are found to have central lacteal vessels and a capillary networks of blood vessels; and so may be spoken of as villi although somewhat reduced in size.

Bird.	Villi near the Pyloric valve small intestines.			Villi near the cæca small intestines.				Villi of the cæca.				Villi of the large intestines.				Total number of villi in the intestines.	Remarks.
	Form.	Size.	Number per sq. mm.	Form.	Size.	Number per sq. mm.	Number in small intestines.	Form.	Size.	Number per sq. mm.	Number in both cases.	Form.	Size.	Number per sq. mm.	Number in large intestines.		
Domestic duck	Thin triangular with sharp tips.	1.2 mm. high by .3 mm. at the base.	12	Thin oblong plates with even edges.	.6 mm. high by .3 mm. at the base.	15	440,000	Somewhat tooth-shaped.	.4 mm. by .3 mm. at the base.	15	15,000	Thin screen shape.	.6 mm. high by .8 mm. broad	5	16,000	471,000	See figures 43–46 and intestinal curve No. 4.
Ruffed grouse..... (Bonasa umbellus).	Thin leaf-like.	1 mm. by .3 mm.	10	Somewhat tooth-shaped.	.6 mm. by 1.3 mm.	4	12,000	I—Villi near the small intestine somewhat columnar and pointed. II—Villi near the tip of cæca low, mound like.	I—.8 mm. by .2 mm. II—.3 mm. by .3 mm.	12–15	8,000	Large tooth-shape.	.6 mm. high by .3 mm. at the base.	6	4,000	24,000	In the last part of the cæca there are a number of rather high longitudinal folds upon, and between which numerous small wart-like villi may be seen. See intestinal curve No. I.
Bob-white ..... (Colinus virginianus).	Thin leaf-like.	.8 mm. by .3 mm.	8	Thin pointed.	.3 mm. by .3 mm.	12–15	7,000	I—Similar to above. II—Similar to above.	I—.3 mm. by .2 mm. II—.1 mm. high, .1 mm. broad	I—12 II—about 100	8,000	Tooth-shaped or somewhat finger-shaped.	.4 mm. high by .1 mm. at the base.	15	1,000	16,000	In the last part of the cæca there are a number of large folds upon and between which small wart-like villi may be seen. See intestinal curve, No. II.
Domestic turkey ..	Thin slender tipped	1.2–1.8 mm. by .4 mm.	12–15	Thin leaf-like.	.8 mm. by .2 to .3 mm.	15	990,000	Leaf-like.	1.3 mm. by .2–.3 mm.	15	66,000	I—Leaf-like in upper large intestine. II—3 cm. from anus, villi low wart-like.	I—.8mm. by .2–.3 mm. at base. II—.3 mm. by .3 mm.	I—15 II—3	54,000	1,110,000	Villi extend down the cæca about 8 cm. leaving the remaining half of each cæcum smooth.

Maryland yellow throat ..... Most of the villi are long slender .4 mm. high by .05–.08 mm at the base.
(Geothlypis trichas.)

Least flycatcher ..... Small almost columnar villi, .3–.4 mm. long by .08 at the base, about 200 per sq. mm.
(Compidonax minimus.)

Yellow warbler ..... Villi somewhat finger-shaped, .1 mm. by .05 mm. at the base. There are about 75–125 per sq. mm.
(Dendroica aestiva.)

Humming bird..... Villi are somewhat columnar but flattened and often sharp tipped. Near the pylorus they are about .6 mm. by .15 mm. broad at the base, and there are about
(Trochilus colubris.) 100 per sq. mm. Near the anus the villi are about .2 mm. by .035 mm. at the base and there are about 150 per sq. mm. In the whole intestine there are about 15,000 villi. (See intestinal curve, No. 3.)

Cedar bird ..... Zigzag folds closely placed, which in some places are broken up into villi .8–.2 mm. high.
(Ampelius garrulus.)

Scoter duck ..... Zigzag folds .8–.1 mm. high by .5–.8 mm. thick replaced in some places by isolated parts or villi.
(Oidemia deglandi.)

Night heron ..... Zigzag folds replaced in places by villi .8–.1 mm. high by .4–.7 mm. broad by .08–.1 mm. thick.
(Nycticorax nycticorax.)

Downy woodpecker ..... Near the pyloric valve villi quite broad, somewhat flattened and sharp pointed, 20–30 per sq. mm., 1 mm. high by .4 mm. at the base. Near the end of the
(Dryobates pubescens.) intestine villi are nearly columnar, .3 mm. long by .8 mm. wide at the base.

Great horned owl..... Near the pylorus villi about .8 mm. high by .15 mm. at the base and flattened columnar in form. Villi in rest of the intestine smaller. The villi continue
(Pubo. virginianus.) 4 cm. or half way into the cæca and the remaining 4 cm. of each cæca has no villi; but very low folds were formed.

Rough-legged hawk ..... The villi are almost perfect cylinders in some places, while in others they are somewhat flattened. The largest are about 1.5 mm. long. In the last part of
(Archibuteo lagopus.) the intestine where the villi are much smaller, their tips are somewhat oblong, and for a short distance are arranged as the villi are in the small intestine of the domestic duck, just above the cæca (Fig. 4). The cæca are only 7 mm. long and filled with large folds.

2. Very long cylindrical or thread-like forms such as are found in ox, opposum, camel and others (Figs. 11-13).

3. Short thick mound-like, or low columnar forms, such as are found in the cæcum of rabbit and the small intestine of horse, although this last very nearly comes under the next heading (Fig. 40).

4. Cylindrical villi, such as are found in cat, dog, raccoon, and some Insectivora and in the ileum of man (Figs. 17-19).

The following table (page 470) gives the results of observations on the form, size and number of villi in some of the mammals which were available for study.

In *Macacus cynomolgus* one of the primates, Rawitz, **94,** describes small villi and larger "ramified" forms. In the intestine of a number of monkeys examined leaf-like villi were observed, and in specimens of Gorilla, Chimpanzee and Orang more or less leaf-like villi were found.

In the intestine of a child at term, the villi are thin, more or less leaf-like or fold-like. Many of these have broad bases and might almost be considered folds. Their height is from .2-.4 mm. by .2-.7 mm. at the base. The villi of a single region varied about as much as the villi of different regions differed from one another. Although the villi in one part of the intestine are very similar to those of other parts in height and general character, a slight difference can be noted, for the region near the pylorus has more fold-like villi than the central part. The central part has more long villi of a triangular shape than has the upper part of the intestine, and near the end of the small intestine there are more villi with their tops nearly as broad as their bases. There are from 10-12 villi per square mm., and in the whole intestine, about 1,000,000 (Figs. 25-28) and (Intestinal curve No. 12).

In adult man, Stöhr describes leaf-shaped villi in the duodenum and cylindrical in the rest of the intestine. Sappey estimates the number of villi in man to be from 6,000,000 to 10,000,000. Others estimate 4,000,000. The greater number of villi in the adult are in the upper part of the intestine, 10-18 per square mm.; farther towards the cæcum, 8-14 per square mm. Many authors have described club-shaped villi in the intestine of man, but when thin sections are made of cylindrical villi which were fixed in some such fluid as picric acid, club-shaped forms are found; so it seems improbable that club-shaped villi in man are typical.

Intestinal Curves of Mammals and Birds.

In the following diagrams (p. 471) the relative height of villi is indicated by the distance of the continuous curved lines above the heavy

Animal.	Villi of the small intestine near the pyloric valve.			Villi of the small intestine near the center of the intestine.			Villi of the small intestine just above the cæcum.			Total.	Remarks.
	Form.	Size.	Number per sq. mm.	Form.	Size.	Number per sq. mm.	Form.	Size.	Number per sq. mm.	Number of villi in intestine.	
Calf a few days after birth	Thread-like slender cylinders.	.5–.8 mm. long by .1 mm. at base.	18-20	Thread-like.	.4–.6 mm. long by .15 mm. at base.	25	Thread-like.	1 mm. long by .1 mm. at base.	25	20,000,000	See Figs. 11–13, Pl. II.
Domestic rabbit	Thin leaf-like rounded tips.	.5 mm. by .9 mm.	5-6	Thin leaf-like triangular shaped.	.5–.7mm. by .2–.3 mm.	1	Almost thread-like but rather broad base than tips pointed.	.6 mm. by .1 mm.	30	900,000	In the cæcum there are numerous small mound-like villi. In the small intestine nearest the pylorus there are numerous short folds or fold-like villi .15 mm. high by .8 mm. long. (See Figs. 20–24, Pl. II.)
Mouse (Mus musculus).	Thin leaf-like rounded tips.	.5 mm. by .3 mm.	20	Thin leaf-like triangular shaped.	.35 mm. by .15 mm.	40	Thin points almost cylindric; slender.	.1 mm. by .03 mm.	40	130,000	See Figs. 34–39, Pl. II, and intestinal curve, No. 9.
Muskrat (Fiber zibethicus).	Oblong, thin, flat with sq. ends.	1.4 mm. by .4 mm.	10	Oblong, thin, flat with sq. ends or nearly so.	.8 mm. by .3 mm.	10–12	Oblong thin flat plates.	.4 mm. by .2 to .3 mm.	10	500,000	See Figs. 14–16, Pl. II, and intestinal curve No. 8.
Grey squirrel (Sciurus Carolinensis).	Thin leaf-like.	.3 mm. by .4 mm.	5–8	Thin leaf-like triangular shaped.	.2 mm. by .1 mm.	20	Thin, slender, pointed.	.5 mm. by .1 mm.	30	Not determined.	
Porcupine (Erethizon dorsalis).	Thin leaf-like without rounded edges.	1.4 mm. by .6 mm.	5–7	Thin leaf-like triangular.	.3 mm. by .3 mm.	10–12	Thin, slender, pointed.	1 mm. by .3 mm.	12–14	20,000	Near the pyloric valve there are a number of little folds with wavy edges. (See intestinal curve No. 11.) The greatest extent of one of these is 3 mm.; besides these small wavy folds which look much like villi pointed at their bases, there are few rather low thin straight folds 5–10 mm. long, .1 mm. thick and 1 mm. high.
Adult domestic cat (From several specimens).	Cylindric.	.5 to .7 mm. high by .1 mm. at the base.	15	Cylindric.	.3 mm. high .2 mm. at the base.	20	Cylindric.	.5 mm. h. by .06 mm. at the base.	25	1,000,000	See intestinal curves Nos. 5 and 6 and Figs. 17–19, Pl. II.
Half grown cat	Cylindric.	.5 mm. by .08–.1 mm.	15	Cylindric.	1.8 mm. by .15–.18 mm.	20	Cylindric.	.3 to .5 mm. by .06 mm.	25	600,000	The smaller number of villi in this animal is largely due to the smaller size and length of the intestine than that of an adult cat.
Adult domestic dog	Cylindric.	.8 mm. by .2 mm.	12–15	Cylindric.	.3 mm. by .25 mm.	15–20	Cylindric.	.7 mm. by .15 mm.	20–25	2,000,000	
Domestic dog a few days old	Cylindric.	.6 mm. by .1 mm.	15	Cylindric.	1.2 mm. by .2 mm.	20	Cylindric.	.4 mm. by .1 mm.	25		Throughout the small intestine there was a great number of much smaller villi; .2 mm. high by .05 mm. thick; these small villi are probably the beginnings of new villi which are formed since birth.
Raccoon (adult) (Procyon lotor).	Cylindric.	.4 mm. by .1 mm.	10–12	Cylindric.	.8 mm. by .12 mm.	10–12	Cylindric.	.3 mm. by .05–1 mm.	15–20	190,000	As there is no cæcum the villi described under the last three columns are from near where the villi end or 25 cm. from the anus.
Raccoon (half grown). (Procyon lotor).	Cylindric.	.3 mm. by .1 mm.	20–25	Cylindric.	.4 mm. by .1 mm.	30	Cylindric.	.1–.2 mm. by .05–.08 mm.	40	125,000	Villi described under the last three columns are from where the villi end or 16 cm. from the anus.
Opossum (Didelphys virginiana)	Thread-like slender cylinders.	3 mm. by .1 mm.	5-6	Thread-like slender cylinders.			Thread-like slender cylinders.				Near the cæcum the villi are smaller but much like those of the pyloric region in shape.
Shrew-mole (Blarina brevicauda)	Cylindric.	.5 mm. by .1 mm.	50–80	Cylindric.	.3 mm. high by .06 mm. thick.	80	Cylindric.	.2 mm. by .04 mm.	80	180,000	The last three columns are where the villi end or 3 cm. from the anus. (See Figs. 32 and 33, Pl. II.)

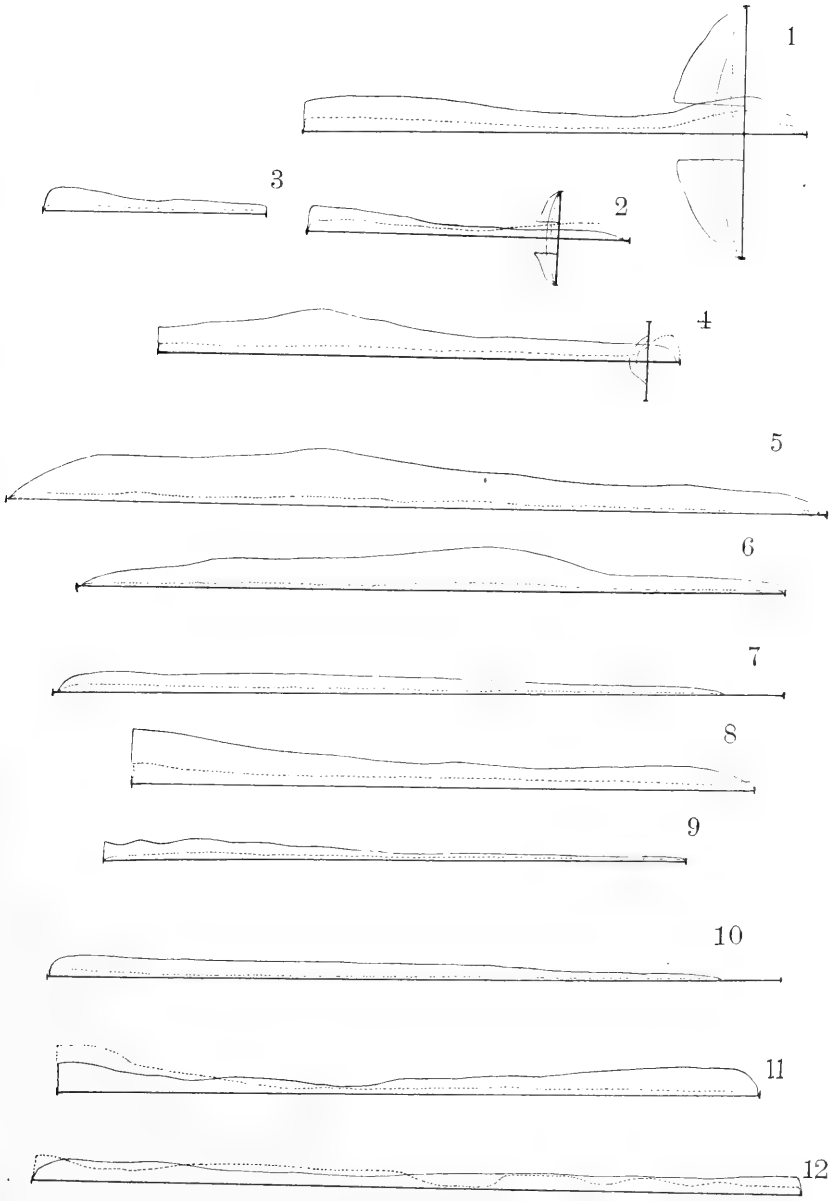


Fig. 1. Ruffled grouse
Fig. 2. Bob-white
Fig. 3. Humming bird
Fig. 4. Domestic duck
Fig. 5. Old cat
Fig. 6. Young cat
Fig. 7. Bat
Fig. 8. Muskrat
Fig. 9. Mouse
Fig. 10. Shrew mole
Fig. 11. Porcupine
Fig. 12. Homo at term

base line and the relative breadth of villi by the distance of the dotted lines from the base line. The left hand of each diagram represents the cephalic, and the right the caudal end of the intestine.

In Figs. 1, 2 and 4 of birds, the cæca are indicated by the heavy perpendiculars above and below the base line. All of the base line to the right of these perpendiculars indicates the small intestine.

In Figs. 1 and 2 large folds in the cæca are indicated by the curved lines which are the farthest from the base line.

THE DEVELOPMENT OF FOLDS AND VILLI.

The development of folds and villi may be considered in two ways:

1st, by the study of the different stages found in adult species.

2nd, by the study of their embryological development in one or more animals.

By a brief review of the typical forms found in some intestines described, it may be seen that in a simple type of intestine only slight longitudinal folds occur. In some other species there are rather more prominent, nearly straight folds; in others, wavy folds are found, and in some others very wavy or zigzag folds may be seen. In a few species, net-arranged folds are observed.

The investigations of recent years and the researches described in the present paper, point unmistakably to the conclusion that both phylogenetically and ontogenetically villi are developed from mucosal folds. The phylogenetic development may be illustrated in two ways by various groups and may be made out quite well in animals where folds and villi occur in the same intestine.

The simplest way in which villi are developed from folds is their formation from long, very zigzag, longitudinal ones, such as described in the blacksnake, sea gull, night heron, cedar bird, etc. Villi seem to be formed by simple separations at the angles of the folds. In several forms of animals, such as the wild duck or the night heron, zigzag continuous folds are found on one part of the intestine and in other places the villi are arranged in zigzag rows; these rows look like folds but are broken up into villi. In some intestines, such as those of the chicken or the turkey, villi are found which are not regularly arranged and no folds occur in the adult.

Another less common method of development of villi is the development from the net-formed folds. In this method probably very zigzag folds unite with each other, making a rather even network; later in

development this network becomes broken up into a more uneven arrangement by growth of the intestine, and the connections of meshes come to be separated; so here and there villi begin to be isolated from the folds and possibly, also, by farther unequal growth, villi come to entirely replace the folds. This method, although undoubtedly occurring in some forms, as in Amia where all grades of such development are shown, is probably not so common as the development from zigzag folds. In birds, for instance, it seems to be rather rare. Development, as shown by embryology, is very necessary to confirm the rather scattered results of the study of adult conditions.

Development of Villi in the Chick.

Plates III, IV and V, Figures 47-58 and 60-70.

There is a great variation noticeable in the intestines of chick embryos; even those of the same size and same age may differ much in regard to the development of the mucosa. In the following discussion the time of incubation is taken as a criterion of age and only those embryos are described which seem to show typical stages of development.

In some early embryos different stages of development may be found in different portions of a single intestine, therefore it might seem possible to obtain all stages from one intestine, but this is only true to a certain extent; so specimens of different ages are necessary in order to determine exactly what takes place in all parts of the intestine.

All parts of a seven day chick's intestine appear without folds or other elevations of the mucosa.

At eight days' incubation, rather large longitudinal folds of the mucosa make their appearance in the pyloric part of the small intestine. A cross section of the intestine at this place usually shows about three large folds (Fig. 48). These folds continue for some distance (Figs. 60, 61), but as the caudal portion of the intestine is approached they decrease in size and number and the last of the intestine shows no folds (Fig. 47); or the typical condition of earlier embryos.

In the small intestine of a nine-day chick there are seen to be six to seven folds which are somewhat smaller than those of the eight-day chick. They are also much more distinct. These folds are parallel, longitudinal and nearly straight (Fig. 49, A and B, and Fig. 62). Towards the cæcum these folds are less numerous and in the last part of the small intestine they are entirely absent.

In the duodenum of a ten-day chick, there are from 9-16 longitudinal folds which vary somewhat in size. Some are low and rather shorter than others. Most of the folds near the pylorus are rather wavy from side to side, but some are nearly straight. Farther along in the small intestine the folds are less numerous or entirely absent.

In the small intestine of an eleven-day chick the longitudinal folds near the pylorus have become quite wavy from side to side (Fig. 50 A and B, and Figs. 63-64). Farther towards the cæca they are less numerous and not so wavy; still farther along there are only about ten straight folds; and next the cæca there are a few folds or mound-like elevations, shorter and thicker than the others.

In the small intestine of a thirteen-day chick the folds are found to be very zigzag, the angles made by the folds are quite sharp; the tops of these folds are more or less divided, that is, partially broken up into villi (Figs. 51, 52). Lower in the intestine, similar folds are observed for some distance, or in places irregular elevations of epithelium which are possibly short folds. Towards the cæca the folds nearly disappear.

In a chick of fourteen days almost perfectly formed villi are found in the duodenum (Fig. 53). In sagittal section these villi are seen to be united at their bases, so they form zigzag folds, partly divided into villi at the free edges (Fig. 54). Villi have been formed in part by folds becoming very zigzag, the epithelium of the sharp fold angles is in this way gradually brought in contact and then separation into villi takes place from the tips downward (Figs. 69 and 70).

Lower in the small intestine less advanced conditions of folds are found, but just above the cæca the conditions noticed cephalad are much less evident and it is very probable that villi are formed here, without going through all the fold stages.

In a fifteen-day chick there was found a remnant of the zigzag arrangement of villi; short folds were noticed similar to the larger ones of earlier embryos.

In the small intestine of a sixteen-day chick there are true villi and the zigzag arrangement is absent throughout.

Up to about ten days of incubation there are no folds in the cæca, but at ten days a few rather large irregular folds appear. At eleven days a few large thick elevations may be seen, but most of each cæcum is bare of folds of any sort. At thirteen days the cæca have thick irregular mounds (Figs. 55 and 67). These elevations grow upward irregularly so that some parts outstrip others; as growth proceeds, the elevations become divided into smaller and higher projections and at fourteen days these may be spoken of as low villi of various sizes,

thickly placed and irregularly distributed (Figs. 56 and 66). The further growth and multiplication of villi in the cæca was found to be the same as in other parts of the intestine. The development of villi in the large intestine does not begin until eleven or twelve days of incubation, then small folds occur in some places, these may go on growing and help to form villi in the way in which they are formed in the cæca, but later stages show very little signs of villi formed as they are in the cæca. Thirteen- or fourteen-day chicks show quite uniformly throughout the large intestine, very small elevations which begin at first as simple elevations of epithelium, later a connective-tissue core seems to push up into these little elevations and true villi are formed by further growth (Figs. 58 and 65).

The development of villi in chick may be summarized as follows:

At first the intestine is entirely free from elevations of any sort. Later, rather large parallel folds of the mucosa gradually make their appearance in the small intestine; first near the pylorus. These folds soon become more numerous, the large folds divide into smaller ones and rather small new folds are developed between those originally formed; and in some cases these later folds also pass through all the stages which the earlier ones do. The slightly wavy folds become more wavy, first in the pyloric part of the intestine, and later more caudad; this wavy appearance becomes more and more marked until those which are at first slightly zigzag grow very zigzag. The angles become very acute and epithelial cells from both sides of many of these begin to nearly touch each other in the centre of the folds. This last is especially true of the tips of the folds. Villi are later formed by unequal growth and separations at the sharp fold angles from the tips downward. This separation begins when the folds are first very zigzag; the complete separation into villi occurs quite late in some parts of the small intestine, and long after the first folds are developed, villi make their appearance between other villi, without passing through a fold stage. In the lower part of the small intestine, many of the stages noticed in the upper intestine are entirely omitted and villi are developed, either from short irregular elevations, or as simple upgrowths of the mucosa.

After all traces of folds are lost the villi grow very rapidly in height and new villi are formed as simple upgrowths of the mucosa. Villi in the cæca do not make their appearance until some time after their beginnings are formed in the small intestine. They are formed from thick irregular elevations or short mucosal folds which gradually become divided into smaller but higher projections by their own unequal growth.

In most cases the large intestine remains without villi for a longer period than the cæca; here the thick irregular elevations similar to those in the cæca are seldom found. In the large intestine numerous very small processes composed entirely of epithelial cells make their appearance, connective-tissue cores penetrate into these projections and in this way villi are formed without passing through a fold stage. Later villi develop as simple upgrowths of the mucosa, probably without being preceded by the small, purely epithelial processes.

The Development of Villi in Mammals.

Plate VI and Fig. 59.

The development of villi in mammals has been recently studied by Voigt, 99, and Berry, 1900. Voigt speaks of villi formed in pig from large elevations of the mucosa which gradually become broken up by means of depressions; from these elevations villi grow up. Berry describes a similar method of villus formation in man, but he shows a little more clearly that the first elevations of the mucosa are in the form of rather large, irregular, longitudinal folds; these folds become broken up into villi.

The following presentation of the development of villi in mammals is confined to the intestine of the white rat.

As no idea could be obtained in regard to the age of the embryos, size alone is given, although this often expresses very little about the stage of the embryo's development.

Nowhere near the amount of material was available as in the study of chick, but in a way it was not required because the development of the villi is more direct and uniform than in chick.

The first indications of folds in the intestine of the white rat was seen in embryos of about 16-20 mm. from the base of the tail to the tip of the snout. The first folds are usually made up of very thick epithelial masses, these are somewhat short and generally run parallel, with the lumen of the intestine (Fig. 75). Into some of these very early folds a developing core of connective tissue may be seen. In an embryo of 33 mm. there were several large, thick, rather regular and parallel folds with well-marked connective-tissue cores; there were four near the pylorus which nearly filled the lumen (Figs. 76 and 77), two were large and two smaller, but after extending parallel for about 1.5 mm. there were only three folds and 1 mm. farther caudad only two folds and soon these ended (Figs. 71 and 72).

No wavy or zigzag folds were found in any of the specimens but in every case, villi either developed from parallel or irregular folds (Figs. 73, 74 and 79-80), or arose entirely without folds.

An embryo of 34 mm. which was much more advanced than the one of 33 mm., showed the lower parts of the intestine more or less free from folds, but in most of the intestine various stages of folds were found partly broken up into villi (Fig. 73). In some cases villi appear to be formed without passing through a fold stage, but usually, as in different parts of the intestine of this embryo, villi were formed from folds, that is, these folds grew higher and became divided into smaller but higher portions by irregular and unequal growth, and these projections soon grew like the villi of the adult.

The large intestine of the white rat is rather backward in its development, villi are not formed until the embryo is 40-50 mm. in length and then as in chick, the villi first appear as solid epithelial processes into which later the connective-tissue cores extend. Such a development of villi has been described in the large intestine by Brand, 77, and Patzelt, 82.

In the white rat the development of villi may be summarized as follows:

Folds are first formed near the pylorus; they are rather large, regular and parallel. Similar folds develop later in other parts of the intestine, but in some places no folds are formed. By unequal growths the folds gradually break up into villi and new villi are also developed without passing through a fold stage (Fig. 57). The beginnings of villi in the large intestine may be first recognized as little elevations of epithelial cells (Fig. 59). A core of connective tissue pushes up into these cell masses and the villi grow upward and become like those in other parts of the intestine.

The development of villi seems to take place rather early as compared with chick and the zigzag fold stage so characteristic of birds is lacking.

Disappearance of Villi from the Large Intestines of Mammals.

(Figures 81-87.)

It is well known that villi occur uniformly in the small intestine alone in adult mammals, but there are a few exceptions. In the cæcum of adult rabbits there are a number of very low mounds which are

found to contain a central lacteal surrounded by the usual villus network of blood vessels. These little elevations resemble the villi of the duodenum more closely than any of the other villi of rabbit and they are without doubt true villi, although much reduced in size. In some animals, such as raccoon, where no cæcum is present, only the lower part of the intestine is free from villi.

Excluding the forms mentioned above, mammals may be said to have no villi in the cæcum or large intestine of the adult.

Villi are usually present in birds and in the majority of forms where they occur they are found throughout the small and large intestine including the cæca, although in some cases the lower large intestine and tips of the cæca present variations in this respect.

Although villi do not occur in the large intestines of adult mammals, they do occur in the intestines of advanced embryos;[4] and in some cases where the young are born in a very immature condition, villi persist for some time after birth. The mammals in which villi are found in the large intestine for a short time after birth, have not been determined to any extent, but at least two forms in which this is the case are rabbit and white rat. Examples of intestines where villi disappear before birth are those of man and cat.

In a consideration of the way in which villi disappear in the large intestine of mammals, the following will be confined to their disappearance in the white rat. In this form they persist for a considerable time after the rats are born, and are not entirely absent from the large intestine until 12-14 days after birth.

Before starting with the discussion of the manner in which villi disappear, it will be necessary to speak of the development of the crypts of Lieberkühn, for it has been assumed that there is an intimate relation between the development of the crypts of Lieberkühn and the disappearance of villi.

Lieberkühnen crypts occur in the adult scattered irregularly in the small intestine. They are especially numerous in the ileum, but in the large intestine including the cæcum, they are so numerous that their mouths take up a large part of the intestinal surface. These crypts are simple or occasionally two-branched tubes or follicles of epithelium extending down into mucosa and usually nearly reaching to the muscular coats of the intestine.

[4] An interesting point in regard to the intestine of man which has apparently hitherto escaped observation is the fact that villi are found at one time in the vermiform appendix.

These glands of Lieberkühn were first described by Malpighi in 1688 and later by Lieberkühn in 1745.

The occurrence of these structures in fishes is doubtful, in Amphibia and Reptiles there are small areas where new epithelium is formed, or in other words, centres of cell generation; these little areas may be homologized with the crypts of higher forms. In birds there are crypts which are similar to those of mammals in all respects except size.

As to the manner of their development there have been many different interpretations by the investigators who have given them attention. Voigt, 99, summarizes the work which has been done on the development of these crypts, so a short review is all that is necessary to repeat before beginning the description of the crypts in the white rat.

Kölliker, 61, describes Lieberkühnen crypts in the beginning as deep out-growths of the epithelium, that is, down-growths.

Barth, 68, thought that the glands did not arise as down-growths (Ausstülpungen) of the epithelium, but that under the epithelium was a mesodermal layer from which villi and glands were developed. In the large intestine a similar development was said to take place.

Brand, 77, arrives at the following results: In the large intestine between the papillæ which develop from the surface, the glands are slowly formed. In the small intestine the villi are thickened at the bases, these touch and unite with each other and so separate walls appear, so the tube-like hollow Lieberkühnen glands are formed.

Kölliker comes to express similar views in his text-books of 79 and 94, and later editions of his works give the development much as the following.

O. Schultze, 97. In the stomach and large intestine, papillæ or villi are described which unite by means of low folds, so that little dimples appear, from each of these a deep insinking or end of a gland appears. Later these connecting folds or borders reach to one-half the height of the villi; so now the surface of the intestine appears as a honeycomb. At last the binding folds reach the top of the villi and at their disappearance the whole mucous membrane has the appearance of a honeycomb, in the mucosa are numerous glands which completely fill up the space.

In the small intestine there is a similar formation except that in the depths between the villi, the surface of the mucosa furnishes net-formed, binding folds and the epithelium from the so arising depressions push in short hollow sprouts. The gland formation is not so striking here because the villi during the formation of glands do not dwindle, but on the contrary, become longer.

Whether these depressions and epithelial processes further develop together or whether the epithelial tube later develops in the depths, remains undecided, but it is probable that earlier or later these epithelial tubes develop in the depths.

Patzelt, **82,** studied the large intestine; he recognized small elevations of high epithelial cells, the anlages of later villi. Between these, in groups, short broad epithelial cells with basal nuclei, the anlages of the Lieberkühnen crypts, so that these lie in small depressions between them. Later between the villi, connective-tissue folds are elevated, the villi are in this way bound together so the crypts of Lieberkühn are formed by elevations of the wall, not at all or but slightly by deep growths.

Minot, **92,** describes the Lieberkühnen glands as hollow outgrowths of entoderm extending into mesoderm. Kollmann, **98,** describes the crypts of Lieberkühn as developed between the villi, making insinkings into the depths of the mucosa.

Voigt, **99,** has studied the intestine of pig and comes to results contrary to those of Brand and Patzelt. He describes the intestine at first smooth, then furrows appear which divide the mucous membrane up into more or less isolated elevations and from these elevations villi grow upward, while from the furrows which form a network of connected canals, the crypts of Lieberkühn grow downward as hollow sprouts. Any development of the villus base to form the crypts of Lieberkühn, as seems possible, does not take place.

The intestine of the white rat is in some respects rather unfavorable for the study of the crypts of Lieberkühn, although it is more favorable for the study of the disappearance of villi.

An embryo of 43 mm. in length has crypts in the lower large intestine while no crypts were made out in the small intestine, although the villi were well formed. About the time of birth crypts begin to make their appearance in the small intestine and beginnings may be made out as little areas which appear like slight thickenings or depressions of epithelium, scattered irregularly over the surface of the intestine (Fig. 81). It often happens that one of these crypts begins near a villus base, but this seems to be purely fortuitous (Fig. 83). From birth onward the crypts become more numerous, as do the villi, the latter grow up as little buds of epithelial cells (Fig. 57). The crypts, on the other hand, begin as slight thickenings or slight depressions which vary somewhat in character, but are not sharp depressions at first; later they become more marked and after about five days are like little flasks with narrow necks and small lumens (Figs. 83-86). This character they keep

for some time, later they grow downward and at about three weeks after birth they have the characteristic appearance of long, narrow crypts and appear like continuations of the bases of the villi in the submucosa (Fig. 87).

The development of the crypts in the large intestine is much more clearly seen in some cases, because as development proceeds fewer new villi are formed than is the case in the small intestine. The development of the crypts in the large intestine was found to be similar to those farther cephalad, but apparently we have a sort of compensatory development as follows: In the small intestine few glands are developed between the primordial villi, but as the intestine proceeds with development new villi (such as Fig. 57) are developed between the already formed villi and glands, but in the large intestine after the first villi are developed, there are very few formed from buds, as in the case of the small intestine, and to take the place of such a formation many more crypts are developed than is the case in the small intestine.

So far as my observations go all these intestinal glands develop by a downgrowth of the epithelial surface, similar to that described by Kollmann, 98, and Voigt, 99.

In some cases a crypt may be seen to start at the edge of a villus base, but careful study has convinced me that without doubt the apparent relation of villus and crypt is only apparent, and one may see that in order to have glands composed of bases of villi, it would be necessary to have villi on all sides of a crypt, which is clearly not the case; not only do villi occur which are not near any crypts, but crypts occur which are not near any villi, as can be seen upon looking down upon a bit of mucous membrane from the intestine of a young animal in which some crypts may be easily recognized as well as some others which are just making their appearance (Fig. 81). Such a view shows the young and older crypts very small as compared with the villi and they appear as little circular dark-staining areas scattered irregularly over the mucous membrane. Comparatively few at an early stage are near villi; later as more glands and villi develop it more often happens that we find villi and glands very near each other.

In early stages Patzelt and Voigt recognized the anlages of the Lieberkühnen crypts as little thickened areas of cells between the villi; these areas take a deeper stain and so may be recognized. In white rat these are difficult to observe because they do not stain much more deeply than other parts of the epithelium, but after careful observation these beginning crypts may be recognized first by slight thickenings, then depressions, which take a little darker stain in some cases, due

to the crowding together of a number of nuclei. In later stages the crypts are more easily recognized and they often take a deeper stain than other parts of the epithelium, and because they extend deeper into the submucosa than the simple spaces between villi. This last distinction of course only becomes evident as glands have passed their earliest stage (Fig. 83).

The white rat is better adapted for a study of the disappearance of villi from the large intestine than many other mammals because in this form the villi persist some time after birth, twelve days or more, and so may be studied in all stages.

Villi begin their disappearance early. Just before birth the lower large intestine is entirely free from villi and glands occupy nearly all the surface of the rectum.

In white rat, villi are formed first, then glands are developed in the surfaces between villi and then later, villi disappear in the large intestine. This order of development seems to be quite constant and at first sight it appears as though the disappearance of the villi is in some way associated with the development of glands. There is very little literature upon this point. Minot, **92,** states the general view as follows: "Villi also appear throughout the large intestine, but are obliterated there by an upward growth of glands."

As will be remembered Brand and Patzelt believed in the development of glands by means of an upward growth not a downgrowth, and the idea of Schultze and Minot that glands are in part formed by villi, places their opinion midway between the upgrowth and downgrowth theories of gland development; but so far as the disappearance of villi is concerned the upgrowth theories and the views of Schultze and Minot may be said to agree quite closely. Scherman in **98,** studied the degeneration of the villi in the large intestine of guinea-pig and found that the upper four-fifths of the villi were composed entirely of epithelial cells; only the basal fifth was found to contain a core of connective tissue. When the villi begin to disappear by disruption, only this basal part persists and passes into the formation of Lieberkühnen glands. He states that in other mammals there is no real degeneration or disruption although the villi are used in forming the glands.

Voigt, **99,** did not determine how villi disappeared from the large intestine, but found no disruption and no evidence to indicate that villi had anything to do with the formation of crypts. He considered the possible disappearance of villi by means of growth of the intestine, but did not bring forward any proof of this possibility.

A few facts which seem to point to an intimate relation between the disappearance of villi and the growth of the intestine may be given.

In white rat, very few new villi are formed after birth by buds of epithelium in the large intestine, the large intestine and cæcum at first grow very rapidly in length and diameter, but hardly at all in thickness of the intestinal wall, and during this rapid growth villi grow smaller and smaller until finally they disappear (Fig. 82).

The following measurements are given to show the rapidity of growth.

White rat of 5 days. (Length 6.6 cm.) Large intestine 3.3 cm.; cæcum, .5 cm. Villi in cæcum and upper large intestine.

White rat of 7 days. Large intestine, 4 cm.; cæcum, .7 cm. Villi in cæcum *going* but numerous in upper large intestine.

White rat of 8 days. Large intestine, 4.2 cm.; cæcum, .8 cm. Villi in cæcum nearly gone. Villi in the upper large intestine beginning to go.

White rat of 9 days. (Length 7.8 cm.) Large intestine, 4.9 cm.; cæcum, 1 cm. No villi in the cæcum but few in the upper large intestine.

White rat of 10 days. (Length, 7.3 cm.) Large intestine, 4.7 cm.; cæcum, .9 cm. A very few villi in some places of cæcum and a few in the upper large intestine.

White rat of 11 days. (Length, 8.5 cm.) Large intestine, 5 cm.; cæcum, 1 cm. No villi except a very few small ones in the upper large intestine near the folds.

As to the decrease in height of villi, the following is given for the cæcum, a similar decrease takes place in the large intestine.

5 days, longest villus .3 mm. high.
6 days, longest villus .2 mm. high.
7 days, longest villus .08 mm. high.
10 days, longest villus .035 mm. high.

If the glands were formed by part of the villi we might expect the crypts to be much longer after the villi had been obliterated, but as a matter of fact the crypts are not in the least longer just after the villi have disappeared.

The cæcum is a rapidly growing part of the intestine and villi disappear here after they have ceased to exist in the lower large intestine; that part of the large intestine which retains its villi until the last is the large intestine just below the cæcum, here as already shown the villi persist for a long time after they have disappeared elsewhere. In this region in the adult there are numerous nearly longitudinal folds which

run slantingly about the lumen. These folds of mucosa begin some time before birth and grow larger until in the adult they are of considerable size. They are at first largely made up of epithelium with four or more irregular layers of nuclei. These epithelial folds at first contain a small core of connective tissue, but this soon grows larger and the epithelium becomes similar to that of the adult. Glands grow into the core of these folds until in the adult these folds are almost solid masses of glands. These folds radiate from the junction of the cæcum and colon and from one or more thickened centres of intestinal mucous membrane. They are without doubt homologous with the papillæ filled with Lieberkühnen crypts which occur in a similar location in the rabbit's intestine. As to the bearing these folds have upon the disappearance of villi, it seems probable that the growth of these folds inward must have kept the mucous membrane of this region in a more folded condition than elsewhere, and it may be that villi were not so quickly drawn out as in other places. As soon as a villus begins to become of less height, the reverse of the process which takes place in the growth of late villi, it often happens that a crypt starts to develop very near it. Perhaps the growth of this crypt downward may have some influence on the disappearance of the villus, but crypts do not grow down near *every* villus which is beginning to disappear (Fig. 82).

A few of the reasons for connecting the disappearance of villi from the large intestine with the increase in extent of intestinal surface are:

1. The rapid growth of the intestine; the lack of growth of the villi; and the same size of the glands afterwards as before.

2. The glands and villi are independent structures; the glands develop as downgrowths, the villi as upgrowths.

3. Villi of gradually less height are formed as the surface of the intestine increases in extent and in many cases villi may disappear where no glands are near, or a gland or glands on only a small part of the villus base; some of these glands may start in the base of a villus after it is reduced to a small projection (Fig. 27).

The erroneous idea that villi disappear by growing together is very easy to arrive at because in later stages the crypts of the large intestine become so numerous that in sections there is much the appearance of numerous short villi, which are, of course, small portions of mucosa between crypts.[5]

[5] In the lower large intestine of a white rat of four days after birth, there sometimes occur, besides crypts, gutters which on surface views seem to connect the crypts with each other, but on more careful examination it is found that these gut-

Summary.

1. Crypts of Lieberkühn in the white rat develop as simple downgrowths of epithelium without any relation to the villi.

2. Crypts begin to develop after the villi are formed and make their appearance first in the rectum.

3. Villi of the large intestine slowly decrease in size and disappear as the area of the intestine increases. The rectum is found to be without villi first, next the cæcum and last the upper large intestine.

Some General Conclusions.

As with other organs, folds and villi in all forms present individual variations, but although great variation is encountered with all species, it is especially noticeable in the lower forms; to take specific examples, the folds and villi of Amia or the folds of the toad vary more among themselves than the villi of calf or cat. .

Another point observed upon other organs is the recurrence of similar structures in widely differing groups, as illustrated by Amia and Bufo. These have very similar conditions of arrangement of the folds. Other examples would be the similarity between the folds of the blacksnake and those of some birds; and the similar folds of catfish and some Amphibia. These widely separated forms which have similar structures may have come to such conditions, either by similarity of environment, that is character of the food; or these types may simply indicate that all forms develop similarly and that several widely differing forms may have developed faster and reached a more specialized condition than their near relatives; or that other forms were for several causes retarded in development, and so approximated the less advanced conditions of less specialized animals.

The largest villi were usually found in the larger animals, especially when the species were nearly related. When two or more adult animals of the same species were examined, it was found that the larger animals in most cases had larger villi than the smaller ones, and when young and old animals were compared it was found that these differences were much more striking.

ters, which usually run transversely, are grooves which connect simply the mouths of glands. These occur in the rectum, and in sections the appearance is as if we had crypts with villi overhead and all gradations of villi uniting with crypts and so disappearing. This appearance is easily explained after the study of the surface of the intestine. These depressions are of late origin and are only found long after villi have disappeared.

A typical form of villus is found in the flesh-eating animals. The villi are rather long, thick, cylindrical or finger-shaped, such as found in cat, dog, lion, etc. With insectivorous mammals, this form is slightly modified; the villi may have sharper tips. The villi of insectivorous birds are similar to those of corresponding mammals, but they differ from them in being more flattened. With Raptores, the villi, although more or less flattened, approach the cylindrical or columnar type.

In the vegetable-eating mammals, and some birds past the fold stage, three general types of villi may be recognized:

1. The leaf-like or thin, broad, tongue-shaped form.
2. The long, slender, cylindrical or thread-like form.
3. The short, thick, columnar or wart-like form.

The first sort is the most common and has already been described with some modifications in rabbit, mouse, muskrat and monkey. Also in some birds.

The second or thread-like sort of villus is found in the ileum of some rodents, such as rabbit. This type is found, for example, in the intestines of cow, camel and sea-cow.

The third or thick, columnar to low wart-like forms are found in the cæca and large intestines of some grain-eating birds, as chicken, turkey, partridge, and is also found in the small intestine of horse. These villi differ from the carnivorous type because of their proportionately broader bases, less height, and fewer numbers.

With omnivora, as a usual thing, villi in mammals correspond with either the carnivorous or the herbivorous type; for instance, the raccoon has the carnivorous type and the opossum has the herbivorous type of villus. Seldon do omnivora combine both types in the same animal; however, in man such a condition may be said to exist; that is, there are leaf-like villi in the upper intestine or duodenum and columnar in the ileum.

The development of the first villi in chick very interestingly shows a number of the stages of folds which were found in the adult intestines of lower forms, as well as the conditions existing in a number of adult birds. The much later development of villi in the cæca and large intestine does not show all the fold stages which were found in the small intestine. If these stages were ever developed, there is very little indication of them at present.

The first development of villi in mammals is from straight, parallel folds, no zigzag folds are formed like those in chick. It may be that the zigzag folds were never developed, but it seems at least possible that this zigzag fold stage was once formed, but now entirely omitted, be-

cause villi in mammals are well formed at a comparatively early stage in the embryo.

Brief Summary.

1. Simple folds, villi and valvulæ conniventes involve the mucosa alone.

2. Simple mucosal folds and villi of the intestine are homologous; villi are the more specialized of these and usually occur in higher vertebrates, as mammals and birds.

3. True villi are found in a number of lower forms, although folds of different types are the usual elevations of the mucosa in all classes except birds and mammals.

4. The individual variation in shape, size and number of folds, and villi is marked in all groups, but is less characteristic of mammals.

5. Although a number of divisions of the shape of folds or villi may be made, there are intermediate conditions which connect the different divisions with each other.

6. Four general types of folds are:
   1. Long, straight, parallel.
   2. Wavy, parallel folds.
   3. Zigzag parallel folds.
   4. Net-arranged folds.

7. Four general forms of villi are:
   1. Thin, leaf-like.
   2. Thread-like or long, cylindrical.
   3. Cylindrical or finger-shaped.
   4. Low columnar or wart-like.

8. The largest villi are usually found in the largest animals.

9. The number of villi per square millimeter in many cases is largely determined by the size of the villi.

10. Carnivorous mammals usually have finger-like or cylindrical villi. Herbivora, leaf-like, thread-like or mound-like shapes. Omnivora have either the carnivorous or herbivorous type of villus or both types in the same intestine.

11. By an examination of many species of vertebrates, is is found that the following, in general, represent the steps of specialization:
   1. Few longitudinal, straight folds beginning near the pyloric valve.
   2. Numerous straight, longitudinal folds, more extensive.
   3. Slightly wavy folds throughout the intestine.
   4. Very wavy folds, numerous and thickly placed.

5. Zigzag folds (or net arranged folds).

6. Zigzag arranged villi or otherwise regularly arranged villi; and closely after this:

7. Irregularly arranged villi throughout the intestine.

8. Villi in the small intestine and cæcum, not in the large intestine.

9. Villi in the small intestine alone.

10. Valvulæ conniventes formed.

12. By embryological study, villi are found to develop from folds in the beginning, but later villi may be formed without passing through the fold stage.

13. In chicken embryos straight folds are formed first near the pylorus, these become longer, more numerous and wavy, then zigzag, and then break up into villi at the angles of the folds downwards; later villi lose their zigzag arrangement and others develop without passing through a fold stage.

14. In the white rat the early development of villi is very rapid. In the beginning longitudinal folds appear first in the duodenum, later in other parts of the small intestine. These folds break up into villi and villi are afterward formed as simple upgrowths of epithelium.

15. A study of different species of vertebrates which have folds and villi at the same time in the intestine of the adult, shows two ways in which villi are formed from folds: first, from zigzag folds, the usual way; second, from net-arranged folds.

16. Valvulæ conniventes, apparently found only in man, develop from simple, semicircular thickenings of the mucosa long after the villi are developed. They are simple, secondary mucosal elevations and bear villi on their surface.

17. At one time during the development of the intestines of mammals, villi are found throughout the large intestine including the cæcum and appendix. As the intestine grows in size and extent the villi disappear. The disappearance of the villi seems to be for the most part independent of the development of the intestinal glands or crypts of Lieberkühn.

BIBLIOGRAPHY.

A very complete bibliography of this subject, together with numerous abstracts from many writers, will be found in Oppel's Vergleichenden mikroskopischen Anatomie, Vol. II, 1897.

1868. Barth. Beitrag zur Entwickelung der Darmwand. Sitzungsber. d. Wien. Akad. d. Wissensch., math. nat. Kl., Bd. LVIII, 2 Abt., pp. 128-136.

1900. Berry, J. M. On the Development of the Villi of the Human Intestine. Anat. Anz., Bd. XVII, No. 12-14, pp. 242-249.

1892. Brooks, H. St. J. On the Valvulæ Conniventes in Man. Anat. Anz., VIII, Jahrg., No. 2 und 3, p. 81. 1892.

1819. Buerger, H. Villorum intestinalium examen microscopicum. Sper. inaug. med. Halae, 1819.

1838. Cuvier, G. Leçons d'anatomie comparee. Tome IV, Deuxieme Partie, pp. 171-406. Paris, 1838.

1901. A. Eckers und R. Wiedersheim's Anatomie des Frosches auf Grund eigener Untersuchungen durchaus neu bearbeitet von Dr. E. Gaup. Dritte Abtheilung, Erste Hälfte, Lehre von den Eingeweiden, pp. 91-97. Braunschweig, 1901.

1876. Edinger, L. Über die Schleimhaut des Fishdarmes, nebst Bemerkungen zur Physiologenese der Drusen des Darmrohres. Arch. f. mikros. Anat., Bd. XIII, pp. 651-692. 1876.

1879. Gadow, H. Versuch einer vergleichenden Anatomie des Verdauungssystems der Vögel. Jenaische Zeitschr. f. Naturwissensch., Bd. XIII, N. F. 6, pp. 92-171 und pp. 339-403. 1879.

1891. Gadow, H. In Bronn's Thier-Reichs. Sechster Bd. Vierte Abtheilung. Vögel, pp. 685-713, 1869-91.

1842. Goodsir, J. On the Structure of the Intestinal Villi in Man and certain of the Mammalia, with some observations on digestion, etc. Edinb. New Phil. Jour., Vol. XXXIII, pp. 165-174, 1842.

1866. Grimm, J. D. Ein Beitrag zur Anatomie des Darmes. Inaug. Diss. 4 S. 3 Tafeln, 1866, Dormpat.

1900. Hilton, W. A. The Intestine of Amia calva. Am. Nat., Vol. XXXIV, No. 405, pp. 717-735, 1900.

1900. Hilton, W. A. The Development and Relations between the Intestinal Folds and Villi of Vertebrates. Abstract in Proc. Am. Ass. for the Adv. of Sc., Vol. XLIX, p. 233. 1900.

1892. Kazzander, Jul. Über die Falten der Dünndarmschleimhaut des Menschen. Anat. Anz. Jahrg. VII, No. 23-24, pp. 768-771. 1892.

1879. Kölliker, A. Entwickelungsgeschichte des Menschen und der höheren Thiere, pp. 849-857. Leipzig, 1879.

1899. Kölliker, A. Handbuch der Gewebelehre des Menschen, 6 Auf., Dritter Band, pp. 172-212. Leipzig, 1899.

1898. Kollmann. Lehrbuch der Entwickelungsgeschichte, pp. 343-346. Jena, 1898.

1867. Langer, C. Von. Über das Lymphgefässsystem des Frosches. Sitzungsber. d. K. K. Akad. d. Wissench., math. naturk. Kl. LV Bd., I Abt. pp. 593-636.

1870. Langer, C. Von. Über Lymphgefässe des Darmes einiger Süsswasserfische. Aus. d. LXII, Bd. I, Abt. d. Wiener Sitzungsber. math. nat. Kl., pp. 161-170. 1870.

1870. Leveschin, L. Über das Lymph- und Blutgefässsystem des Darmkanals von Salamandra maculata. Sitzungsber. d. math. nat. Kl. d. K. Akad. d. Wiss. zu Wien., LXI Bd., I Abt., pp. 67-79. 1870.

1857. Leydig, F. Lehrbuch der Histologie des Menschen und der Thiere. Frankfurt, a. M. 1857.

1884. Macallum, A. B. Alimentary Canal, etc., of Amiurus catus. Proc. Canadian Inst. Toronto, New series, Vol. II, pp. 387-417. 1884.

1886. Macallum, A. B. The Alimentary Canal and Pancreas of Acipencer, Amia and Lepidosteus. Jour. of Anat. and Physiol., Vol. XX, pp. 604-636. 1886.

1819. Meckel, A. Über die Villosa des Menschen und einiger Thiere. Meckel's deutsch. Archiv. f. Physiol., V Bd., II Heft, pp. 163-182. 1819.

1819. Meckel, J. F. Über den Darmkanal der Reptilien. Meckel's deutsch. Archiv f. Physiol., Bd. III, pp. 199-233, 1817, und Bd. V, pp. 343-347. 1819.

1897. Minot, C. S. Human Embryology, pp. 758-760. Macmillan Co., N. Y., 1897.

1881. Moreau, Emile. Histoire naturelle des poissons de la France, Tome I. Paris, 1881.

1878. Nuhn, A. Lehrbuch der vergl. Anatomie. Heidelberg, 1878.

1897. Oppel, A. Lehrbuch der Vergleichenden mikroskopischen Anatomie der Wirbelthiere. Zweiter theil, Schlund und Darm., pp. 264-357. Jena, 1897.

1868. Owen, R. The Anatomy of Vertebrates, Vol. I, Fishes and Reptiles; Vol. II, Birds and Mammals; Vol. III, Mammals. London, 1866-68.

1882. Patzelt, V. Über die Entwickelung der Dickdarmschleimhaut. Sitzungsber. der Wiener Akademie, math. nat. Kl., Bd. LXXXVI, 3 Abt., pp. 145-172. 1882.

1885. Pilliet, A. Sur al Structure du Tube Digestif de quelques Poissons de Mer. Bulletin de la societe zoolog. de France, Vol. X, pp. 283-308. 1885.

1824. Rathke, H. Über den Darmkanal der Fische. Halle, 1824.

1897. Rauber, A. Lehrbuch der Anatomie des Menschen, 5 Auf., Bd. II, 2 Abt. Leipzig, 1897.

1894. Rawitz, B. Über ramifizierte Darmzotten. Anat. Anz., Bd. IX, pp. 214-216. 1894.

1881. Robin, H. A. Recherches anatomiques sur les mammiferes de l'ordre des Chiropteres. Annales des sciences naturelles 6e serie; Zoologie, Tome XII. Paris, 1881.

1800. Rudolphi, K. A. Einige Beobachtungen über die Darmzotten. Reil's Arch., Bd. IV, p. 63. 1800.

1896. Ruckert, J. Über die Entwickelung des Spiraldarmes bei den Selachien. Arch. f. Entwickelungsmech., Bd. IV, pp. 298-326. 1896.

1898. Scherman, Daria. Über die Rückbildung der Darmzotten des Meerschweinchens. Verh. d. Phys. med. Gesellsch. zu Würzburg, N. F. XXXII Bd., No. L. 1898.

1897. Schultze, O. Grundriss der Entwickelungsgeschichte des Menschen, pp. 369-371. Leipzig, 1897.

1856. Siebold und Stannius. Handbuch der Zootomie. 2 Theil Stannius: Handbuch der Anatomie der Wirbelthiere, 2 Aufl. 1 Buch: Fischer. Berlin, 1854. II Buch: Amphibien, Berlin, 1856.

1846. Stannius: in Stannius und Siebold, Lehrbuch der vergleichenden Anatomie II Theil. Wirbelthiere von H. Stannius, Berlin, 1846.

1863. Vaillant, L. Memoire pour servir a l'histoire anatomique de la sirene lacertine. Annals des sciences nat. zoologie 4 serie. Tome XIX, pp. 295-346. Paris, 1863.

1854. Virchow, R. Über einige Zustände der Darmzotten. Verhandl. d. Würzburger phys. mediz. Gesellsch., Bd. IV, pp. 350-354.

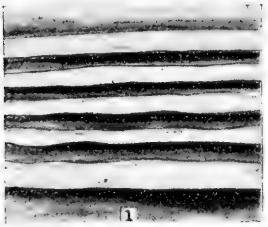
1899. Voigt, J. Beitrag zur Entwickelung der Darmschleimhaut. Anatomische Hefte, XII Bd., I Abt., 38 Heft., pp. 51-68. 1899.

PLATE I.

The following plate is for the purpose of showing some of the types of folds in the lower vertebrates.

The folds are shown as they look when viewed from above after the intestine is cut open longitudinally and spread out flat.

Fig. 1. Folds from turtle. × 5 diameters.
Fig. 2. Folds from Amphiuma. × 5 diameters.
Fig. 3. Folds from the salamander Gyrinophilus. × 13 diameters.
Fig. 4. Folds from a half-grown Necturus. × 5 diameters.
Fig. 5. Folds from Catostomus. × 11 diameters.
Fig. 6. Folds from alligator. × 11 diameters.
Fig. 7. Folds from perch. × 10 diameters.
Fig. 8. Folds from blacksnake. × 5 diameters.
Fig. 9. Folds from toad. × 12 diameters.
Fig. 10. Folds from Catostomus. × 24 diameters.



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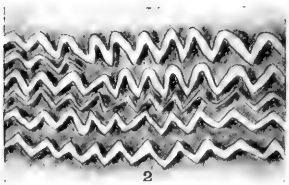

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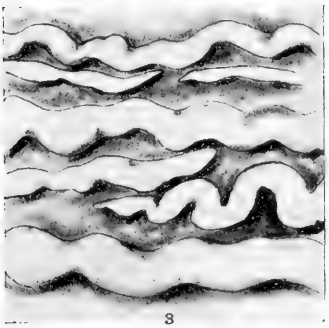

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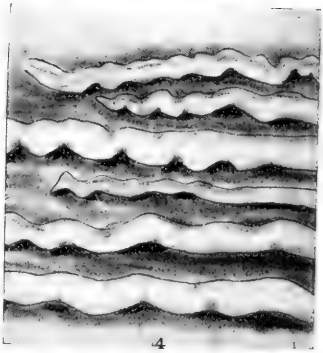

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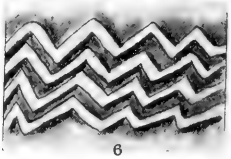

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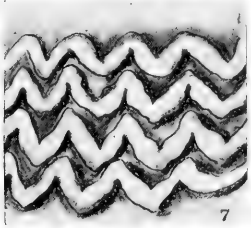

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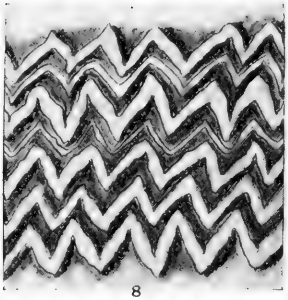

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PLATE II.

The general forms of villi found in mammalia are given in this plate. With the animals in which villi differ greatly in size or form in the different parts of the same intestine, a number of villi are figured.

All figures × 25 diameters.

Fig. 11. Villus of calf near middle of intestine (cylindrical).

Fig. 12. Villus of calf near cæcum (cylindrical).

Fig. 13. Villus of calf near pylorus (cylindrical).

Figs. 14 and 15. Front and side views from villus of muskrat near the pylorus.

Fig. 16. Villus near the cæcum of muskrat's small intestine.

Fig. 17. Villus from middle of cat's intestine (cylindrical).

Fig. 18. Villus from pyloric part of cat's intestine (cylindrical).

Fig. 19. Villus from near cæcum of cat's intestine (cylindrical).

Fig. 20. Villus or fold at pylorus of rabbit's intestine.

Fig. 21. Villus 10 cm. from pylorus of rabbit's intestine.

Fig. 22. Villus 110 cm. from pylorus of rabbit's intestine.

Fig. 23. Villus 190 cm. from pylorus of rabbit's intestine.

Fig. 24. Villus 250 cm. from pylorus of rabbit's intestine, or just above the cæcum.

Fig. 25. Villus Homo at term upper intestine.

Fig. 26. Villus Homo at term upper intestine.

Fig. 27. Villus Homo at term lower intestine.

Fig. 28. Villus Homo at term lower intestine.

Fig. 29. Vilus from bat near pylorus.

Fig. 30. Villus from bat 5 cm. from the pylorus.

Fig. 31. Villus from bat 11 cm. from the pylorus or end of villi.

Fig. 32. Villus from shrew, middle of intestine (cylindrical).

Fig. 33. Shrew, near middle of intestine (cylindrical).

Fig. 34. Villus from mouse 2 cm. from pylorus.

Fig. 35. Villus from mouse 9½ cm. from pylorus.

Fig. 36. Villus from mouse 11 cm. from pylorus.

Fig. 37. Villus from mouse 13 cm. from pylorus.

Fig. 38. Villus from mouse 18 cm. from pylorus.

Fig. 39. Villus from mouse 30 cm. from pylorus or above cæcum.

Fig. 40. Villus from the intestine of horse.

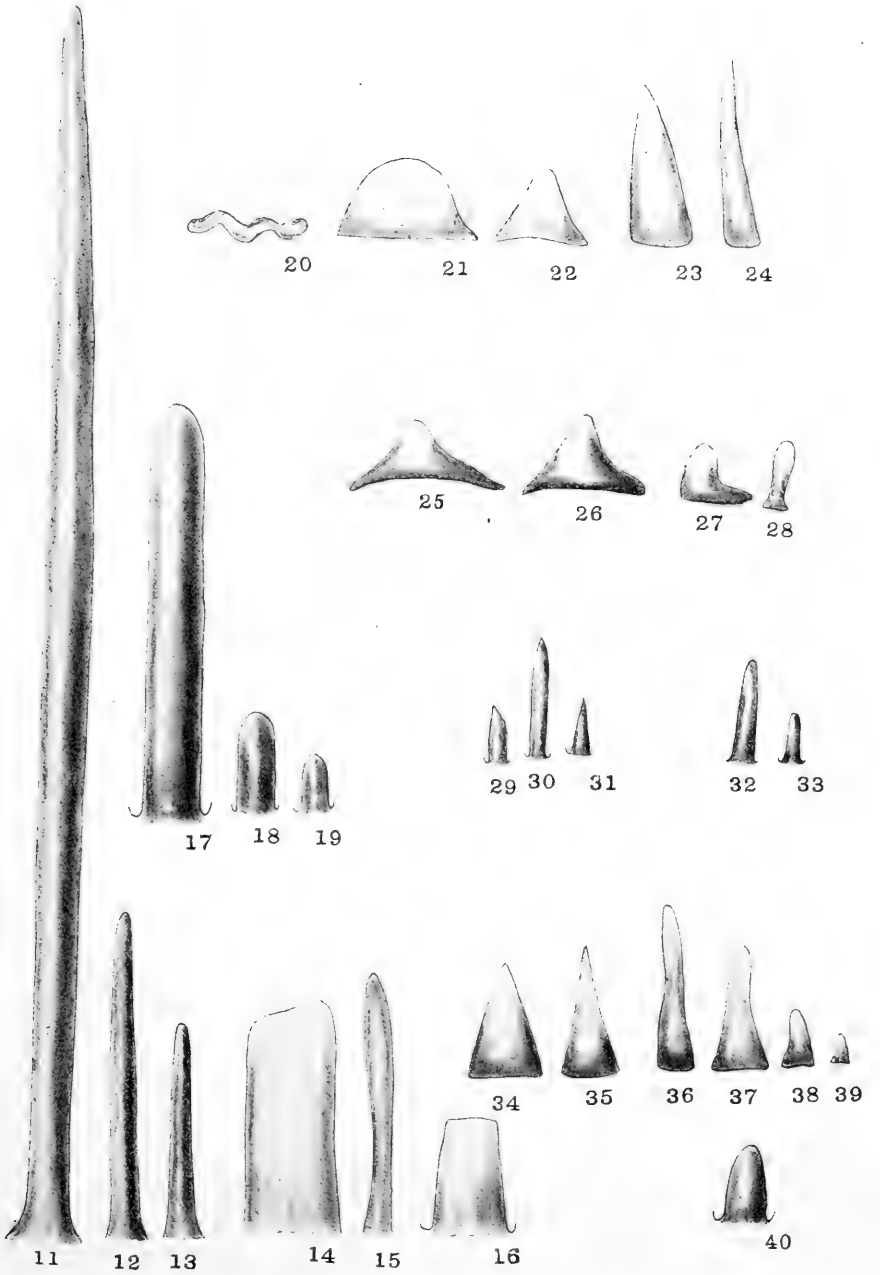
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PLATE III.

Section and surface view drawings from folds and villi of mammals and birds.

Fig. 41. Section across a spiral fold of rabbit's cæcum at time of birth. V = villi, × 50 diameters.

Fig. 42. Section across a developing valvulæ conniventes from a child at term. V=villi, × 50 diameters.

Fig. 43. Villus near pylorus of a domestic duck. × 38 diameters.

Fig. 44. (a) Arrangement of villi 110 cm. from the pylorus of a domestic duck. (b) Villus 110 cm. from the pylorus of a domestic duck. × 38 diameters.

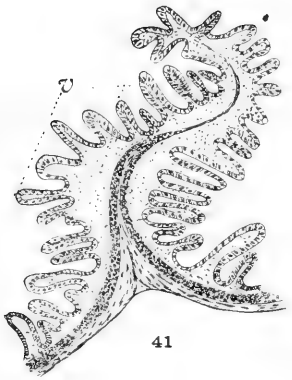
Fig. 45. Villus from the cæcum of a domestic duck. × 38 diameters.

Fig. 46. (a) The arrangement of villi from the large intestine of a domestic duck. (b) Villus from the large intestine of a domestic duck. × 38 diameters.

Fig. 47. Section of the intestine of an eight-day chick, taken near the pylorus. × 88 diameters.

Fig. 48. Section from the intestine of an eight-day chick some distance from the pyloric valve. × 88 diameters.

Fig. 49. (a) Longitudinal section of a single fold from near the pylorus of a nine-day chick's small intestine. × 88 diameters. (b) Transsection from the intestine of the same chick a short distance from the pyloric valve. × 88 diameters.



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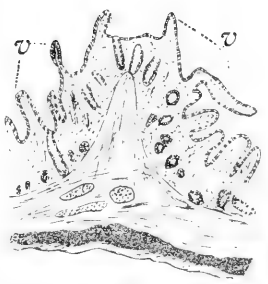

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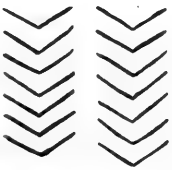

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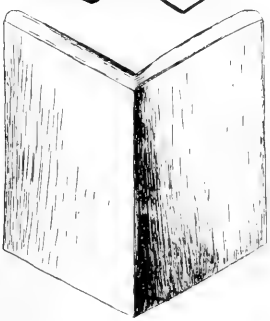

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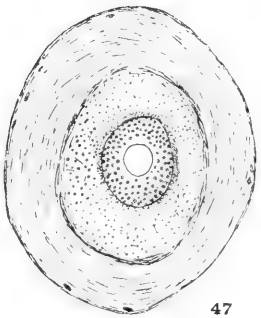

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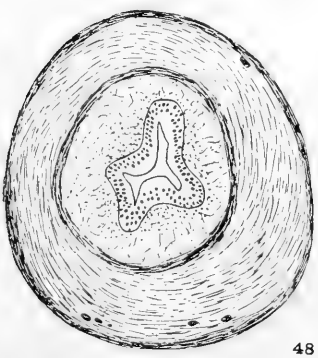

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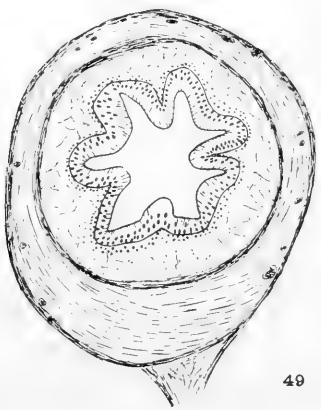

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PLATE IV.

Drawings from sections of embryo chick's intestines.

Fig. 50. (a) Transection of the small intestine of an 11-day chick. × 60 diameters. (b) Longitudinal section through folds from the duodenum of an 11-day chick. × 60 diameters.

Fig. 51. Transection of the small intestine of a 13-day chick. × 60 diameters.

Fig. 52. Longitudinal section through a single fold of a 13-day chick's intestine. × 60 diameters.

Fig. 53. Longitudinal section of the small intestine of a 14-day chick where villi are partly formed. × 60 diameters.

Fig. 54. Longitudinal section of a single fold from a 14-day chick. The larger part of the fold is where the section has cut deepest into the base, and the smaller end is where the top of the fold is cut through at the place where villi are beginning to be formed. × 60 diameters.

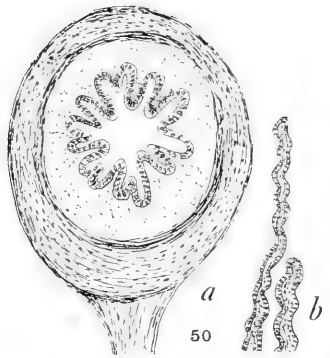
Fig. 55. Longitudinal section of the cæcum of a 13-day chick. × 60 diameters.

Fig. 56. Villi from the cæcum of a 16-day chick. × 60 diameters.

Fig. 57. Villus bud from the small intestine of a 3-5-day white rat. × 300 diameters.

Fig. 58. Section of large intestine of a 14-day chick. × 270 diameters.

Fig. 59. Epithelial projection or villus bud from the large intestine of an embryo white rat. × 417 diameters.



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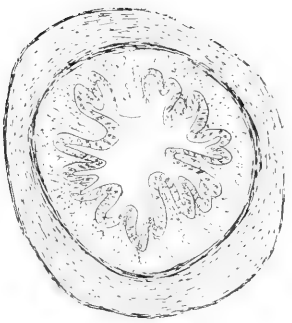

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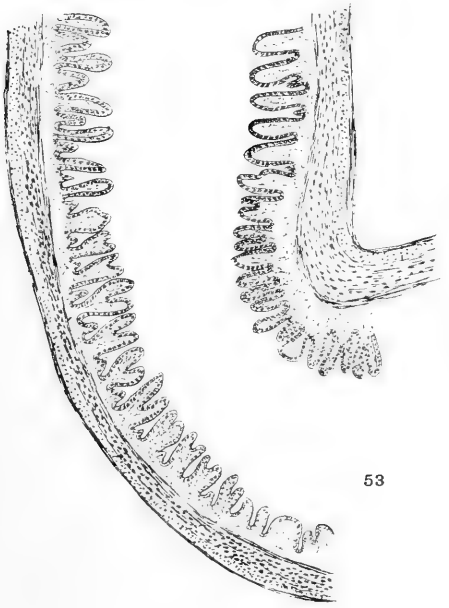

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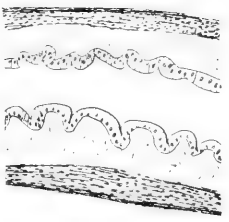

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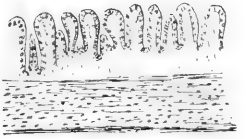

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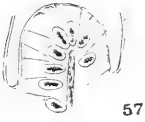

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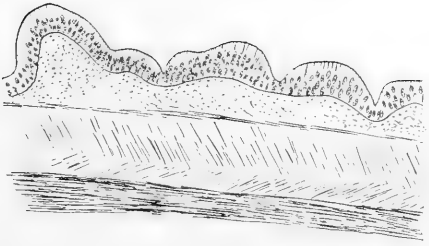

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PLATE V.

Figures of wax reconstructions from the intestine of chick embryos.

Figs. 60 and 61. Folds from 8-day chick. Epithelium alone shown (near the pyloric valve). × 100 diameters.

Fig. 62. Part of intestine from 9-day chick (near pyloric valve). ×50 diameters.

Fig. 63. Part of intestine of 11-day chick, looking almost directly down on folds. × 50 diameters.

Fig. 64. Reconstruction of single fold of 11-day chick. × 100 diameters.

Fig. 65. Reconstruction of intestine of a 14-day chick's large intestine. × 50 diameters.

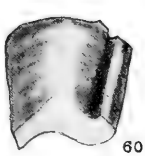
Fig. 66. Reconstruction in wax from the cæcum of a 14-day chick when villi are quite well formed. × 50 diameters.

Fig. 67. Wax reconstruction from cæcum of 13-day chick. × 50 diameters.

Fig. 68. Part of intestine from 13-day chick. Folds quite wavy. × 50 diameters.

Fig. 69. Small 14-day chick single fold near the pylorus. × 100 diameters.

Fig. 70. Reconstructions in wax of a single fold near the pyloric valve from the intestine of an advanced 14-day chick. The fold has begun to break into villi at the fold angles. × 100 diameters.



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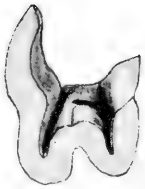

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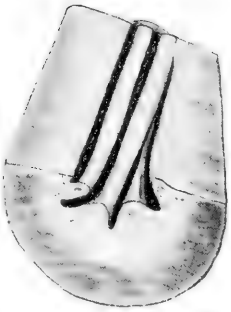

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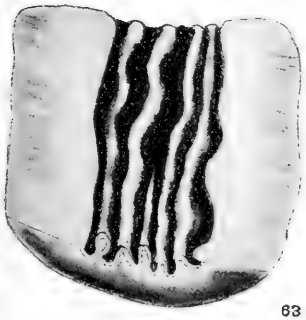

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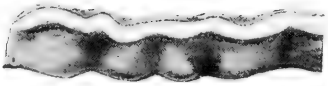

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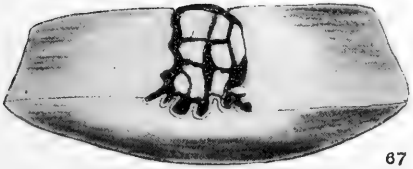

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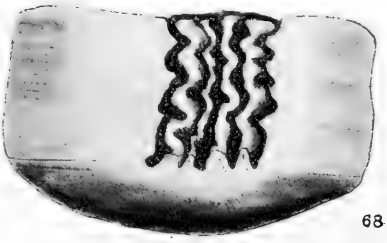

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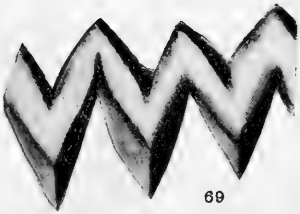

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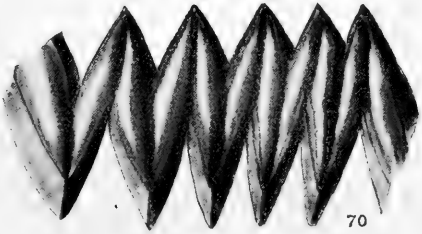

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PLATE VI.

Figures of sections and wax reconstructions from the intestine of embryo white rats, to show the development of folds and villi.

Fig. 71. Cross section of white rat's intestine; length of embryo 3.3 cm. × 56 diameters.

Fig 72. Same as Fig. 61 near the pylorus. × 56 diameters.

Fig. 73. Section of intestine from white rat of 3.4 cm. Villi partly formed. × 56 diameters.

Figs. 74 and 75. Sections of different stages from a white rat of 2.3 cm. in length. × 56 diameters.

Fig. 76. Reconstruction of a single fold removed from near the pylorus of a young embryo of white rat. Thick end of fold is taken nearest the pylorus. × 100 diameters.

Fig. 77. Reconstruction of the rest of the intestine from which the above was taken. The pyloric end of the piece is away from the observer and has one more fold than the end towards the observer. × 100 diameters.

Fig. 78. Portion of white rat's intestine after the folds are beginning to break up into smaller parts. × 133 diameters.

Figs. 79 and 80. Different views of reconstructions showing folds largely divided into villi. × 100 diameters.

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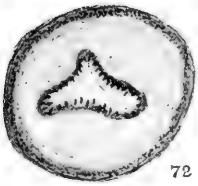

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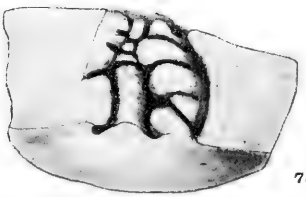

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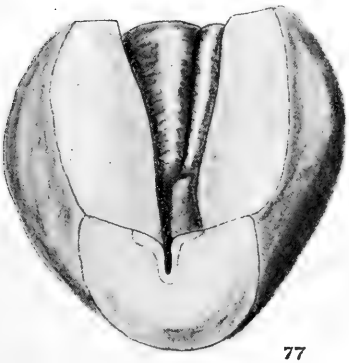

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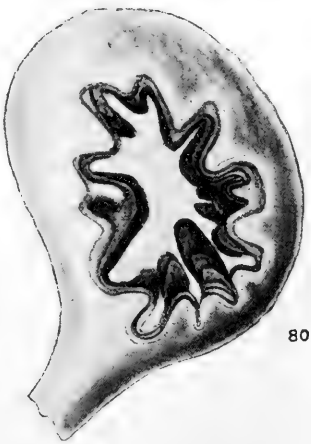

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PLATE VII.

Figures illustrating the development of the crypts of Lieberkühn and the disappearance of villi from the large intestine of young white rats.

Fig. 81. Surface views of villi and crypts from the small intestine of a 3-day white rat. V=villus, c=crypt. × 50 diameters.

Fig. 82. Sections from the large intestines of several white rats to show the disappearance of villi. All the figures × 58 diameters.

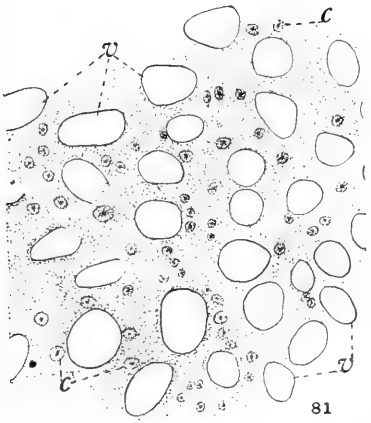
(a) Five days after birth.
(b) Six days after birth.
(c) Eight days after birth.
(d) Eight days after birth, more advanced stage.
(e) Eleven days after birth.

Fig. 83. Villi and beginning crypts from a 3-5-day white rat (after birth). C=crypts. × 300 diameters.

Figs. 84 and 85. Beginning crypts from the small intestine of a white rat 3-4 days after birth. × 300 diameters.

Fig. 86. Crypt of Lieberkühn from the intestine of a 4-day white rat. × 300 diameters.

Fig. 87. Lieberkühn crypt from the small intestine of a white rat, 36 days after birth. × 300 diameters. V = parts of villi, c = crypt and s = surface of the intestine between villi.



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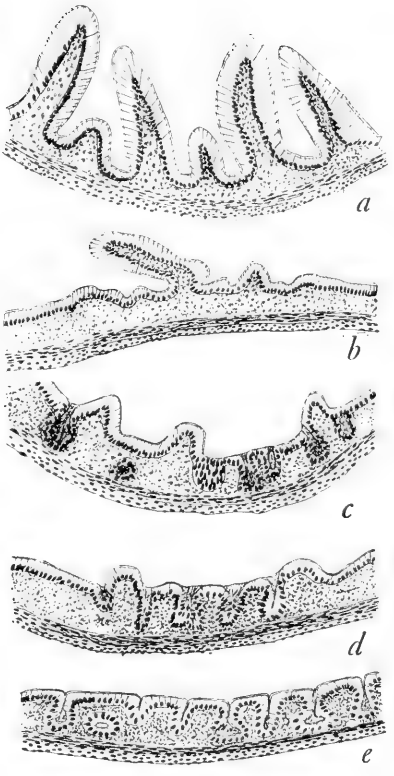

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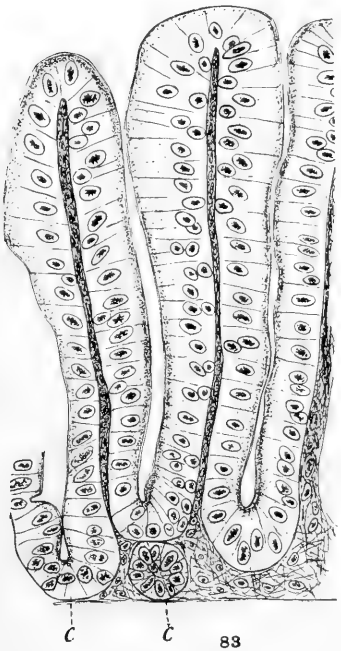

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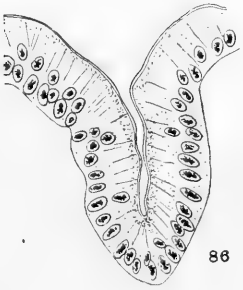

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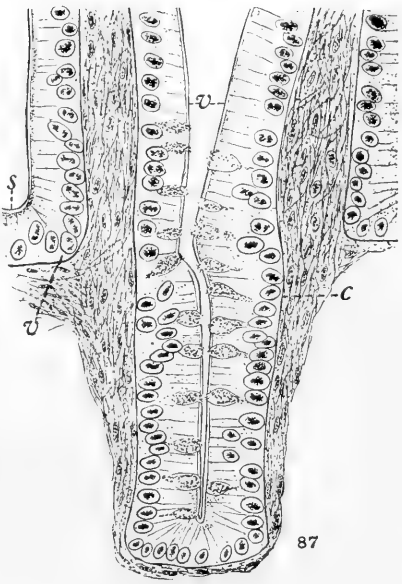

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PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS.[1]

FIFTEENTH SESSION.

University of Chicago, December 31, 1901, to January 2, 1902.

A ONE-YEAR ANATOMICAL COURSE; ITS ARRANGEMENT, MERITS, AND DISADVANTAGES. By Robert J. Terry.

In the course under consideration, the study of anatomy has been limited almost entirely to the first year in order that the students may receive better preparation for the study of physiology and pathology than was the case when these three branches were carried on simultaneously.

Recognizing the fact that the opportunities for learning anatomy well would be lessened by such a change, if other conditions remained the same, the following measures were adopted to obviate the difficulty:

a. The number of subjects taught in the first year has been reduced.

b. The amount of practical work in anatomy has been extended and the work so arranged that every member of the class dissects the same part at the same time.

c. The dissection of fresh organs obtained from the slaughter house accompanies the dissection of the chest and abdomen, so that the structure and topography of these parts are studied together.

d. The order of topics in histology is the same as for gross anatomy, so that for any organ the position and relations and the gross and microscopic structure are gone over during the same period.

Throughout the year a complete record of the dissection is kept and this together with the hospital history is placed on file. The skeletons are carefully prepared and stored. The one-year course has been in operation less than three years, so that a just criticism can hardly be made. The object for which the change was undertaken has, of course,

[1] In future the "Proceedings of the Association of American Anatomists," will be published in an earlier number of the annual volume, as soon as possible after the December meeting.

been obtained, but there is some doubt as to whether the information received in one year will be as permanent as when a longer time was devoted to the study of anatomy.

SECTIONS OF DECALCIFIED BODY. By Robert J. Terry.

As stated by the writer at the meeting of this association in Washington in 1900, where sections of the decalcified body were shown, the ultimate aim of this work is to produce sections thin enough to be useful in reconstructing. The anatomy of the pelvis could be advantageously studied in this way. Imbedding in paraffin was tried, but with little success on account of the imperfect dehydration of the subject. After decalcification, the body (that of an infant) was placed in different strengths of alcohol and at the same time alcohol was injected into the arteries by siphonage. Finally turpentine was used for a bath and also injected. This was continued for a week. For imbedding, the subject was immersed in paraffin kept at the melting point and by a compressed air apparatus paraffin was forced into the vessels. The paraffin imbedding was continued for a week. The results were in the main unsatisfactory.

A CASE OF BREECH PRESENTATION IN A MONKEY. By Robert J. Terry.

Exhibition and description of specimen.

MODELS ILLUSTRATING THE DEVELOPMENT OF THE ARM IN MAN. By Warren H. Lewis. *The American Journal of Anatomy*, Vol. I.

ON THE DEVELOPMENT OF CONNECTIVE TISSUE FIBRILS. By Franklin P. Mall. *The American Journal of Anatomy.* Vol. I.

CERTAIN RACIAL CHARACTERISTICS OF THE BASE OF THE SKULL. By Ales Hrdlicka.

The paper dealt with the middle lacerated foramen, the petrous portions of the temporal bones and the styloid, with demonstration of the different stages of development of these parts in primates and at different stages of life in the whites, and the differences of these parts, fully developed, in the negroes, Indians and whites. In the adult whites, the average middle lacerated foramen is large, the petrous portions appear considerably sunken (bulging of surrounding parts), the styloid is well developed. In the Indian, on the average, the foramen is of but a moderate size, small in the negro, in apes absent; the petrous portions are,

on the average, less sunken in the Indian than in the white, on or almost on the level with the surrounding parts in the negro, bulging more or less beyond these in the primates; the styloid is, in the majority of cases, small in the negro and often small to rudimentary in the Indian. Where the styloid is rudimentary, the vaginal process seems to play frequently a compensatory part. In whites all the mentioned stages of the parts may be observed at different periods of life. Brain development accounts for the differences in the size of the middle lacerated foramen and the relative position of the petrous portions.

CONTRIBUTION TO THE ANATOMY OF THE SCAPULA. By Ales Hrdlicka.

Read by Title.

A NOTE ON THE SUPRACONDYLAR PROCESS. By Robert J. Terry.

In looking over specimens of this variation, it was observed that the process was more or less compressed laterally and that in most cases the spur arose from a longitudinal ridge on the inner face of the shaft of the humerus in its lower part.

A number of supracondylar processes at hand vary in extent from a compressed prong 12 mm. long to a small, sharp projection in the middle of a prominent ridge.

A ridge or line was found in a large number of humeri, in the place where the supracondylar process occurs. It extends downward from, or near to, the principal medullary foramen.

In the literature, Struthers has referred to the line as appearing in most, if not all, human arm bones (*Lancet*, 1863, p. 87). A specimen of supracondylar process with extra head of biceps and high division of the brachial artery was exhibited. The supernumerary origin of the biceps is below the insertion of the coraco-brachialis.

A SKELETON WITH RUDIMENTARY CLAVICLES, DIVIDED PARIETAL BONES AND OTHER ANOMALOUS CONDITIONS. By Robert J. Terry.

A description of the specimen was published in the Journal of Anatomy and Physiology, Vol. 33.

A considerable number of these cases have been reported since Meckel called attention to the anomaly in 1760 (*Mem. de Paris*, 1760). Whenever a careful physical examination has been made, cranial defects have been found present with the abnormal condition of the clavicles.

The deformity has been observed in several members of the same family and in two generations. The functions of the shoulder and arm have been found but slightly disturbed; the presence of the muscle marks on the bones in this specimen indicates at least a normal development of the muscles in this region.

Individuals affected with this deformity are apt to be of small stature, scoliotic, with eyes far apart, the bridge of the nose flat, and the teeth irregularly formed. The malformation is seen in both sexes.

Hultkrantz, J. Wilb., Anat. Anz., Bd. 15, No. 13, p. 237. Carpenter, Geo., Lancet, N. 3932, Jan. 7, 1899.

ON CERTAIN ANOMALIES OF BONES. By George Amos Dorsey.

Read by title.

A SKULL SHOWING AN UNUSUAL NUMBER OF WORMIAN BONES ASSOCIATED WITH IMPERFECT SKELETAL DEVELOPMENT. By Charles A. Parker.

The skull is that of a fairly intelligent man, age 45, a former resident of this city (Chicago), whose spine presented a marked forward dorso-lumbar curvature and the imperfectly developed limbs were curved and misshapen. A progressive recession of the face due to incomplete dentition and a slight circumferential constriction of the cranium with compensating vertical elevation are the only noteworthy mensural variations. The capacity is normal. The noteworthy feature is the extraordinary development and distribution of the Wormian bones, these numbering 172. The whole membranous portion of the cranium behind the coronal suture is composed of these bones. A few in the orbital plates and one measuring 5 x 9 cm. occupying the upper part of the right half of the frontal bone are found in front of this suture. The chondro-cranium contains none. They are most numerous and delicate in the squamo-parietal region, where their slender interlacing processes completely obliterate the usual sutures. They are larger posteriorly and largest in the anterior portion of the parietals whose frontal angles are formed by two symmetrical quadrilateral bones measuring 4 x 8 cm. The sagittal suture is distinct throughout and extends from the nasion to the chondro-skeleton of the squamo-occipitalis.

Note.—This specimen is from the Pathological Laboratory of Rush Medical College, and a more extensive description of the skeletal changes will be published later by Prof. L. Hektoen and the writer.

PRESENT PROBLEMS OF MYOLOGICAL RESEARCH AND THE SIGNIFICANCE AND CLASSIFICATION OF MUSCULAR VARIATIONS. By George S. Huntington.

It was shown that comparative myology and the study of myological variations determine three cardinal facts:

a. Forms which, according to the zoological system commonly accepted, are widely separated from each other, possess identical or very closely allied myological characters.

b. Human muscular variations or supernumerary muscles are frequently homologous with muscles normally present in species apparently very far removed from man in the zoological scale.

c. Within the confines of a single mammalian order, the smaller subdivisions of family and species are frequently sharply differentiated from each other in some details of myological structure, which distinguishes the form possessing the modification from the remaining divisions of the group, no matter how close in other respects their morphological congruence may be. In the light of more complete knowledge, the commonly accepted *relative* phylogenetic position of many forms requires revision. The fundamental mammalian type for any given muscle or muscular group forms the starting point from which the special differentiations of the structure in the various mammalian orders can be traced.

Human muscular variations and supernumerary muscles, as far as they are *reversional* in significance, belong to one of three classes: 1. Archeal Reversions.—Not normally encountered in any mammalian type, but homologous with muscles found in the lower vertebrate classes. 2. Progonal Reversions.—Variations which are not represented by any normal muscle in any *species* composing the *order*, but which are represented by homologous muscles in other mammalian orders. 3. Ataval Reversions.—Reproducing muscular conditions which are abnormal for the species under consideration, but which occur normally in other allied species of the same order.

THE PHYLOGENY OF LONG FLEXOR MUSCLES. By James Playfair McMurrich.

In the lower terrestrial vertebrates the flexor muscles of the forearm terminate at the wrist. The ulnar and radial portions of the original flexor mass early separate to form the flexores carpi ulnaris et radialis, while the median portion which is inserted into the palmar fascia becomes one long flexor of the fingers. This transformation is not due to the extension into the hand of the forearm flexors, but depends partly

on a separation of layers of the palmar fascia and partly on the forearm flexors becoming continuous with palmar muscles.

The flexor profundus is the first to develop by the separation of the deeper layers of the forearm flexor mass together with the corresponding layer of the palmar fascia and muscles which arise from the undersurface of this deep layer of the palmar fascia persist to form the lumbricales. The flexor sublimis later separates from the palmaris longus and carries off another layer of the palmar fascia together with a sheet of muscle tissue attached to this; the muscle tissue degenerates to form the terminal portions of the perforated tendons. The flexor profundus is a flexor perforans because it is the earliest to develop and pre-empts the terminal phalanges.

Contrary to views which have been expressed on the subject, the arrangement of the flexors of the foot seems to represent a more primitive condition than does that of the flexors of the hand.

NOTE ON THE OCCURRENCE AND SIGNIFICANCE OF THE MUSCULUS TIBIO-ASTRAGALUS ANTICUS. By James Playfair McMurrich and R. N. Waterman.

Two cases of the occurrence of this muscle have been described by Gruber, one by Hyrtl, one by Macalister and one by Wagstaffe. The last was found in connection with a defect of the fibula, as was also the case now under consideration. In this the fibula was represented by a slender rod of cartilage except at the malleolus, which was ossified, and the muscle arose from the outer surface of the lower end of the tibia and passed distally behind anterior tibial vessels and nerve to be inserted into the astragalus.

The muscle has usually been regarded as belonging to the Tibialis anticus, but its relations to the tibial vessels which are interposed between it and the Tibialis seem rather to suggest its association with the extensor muscles. It may possibly be regarded as a portion of the extensor mass which has not undergone an extension into the foot.

THE NUCLEAR CHANGES IN THE STRIATED MUSCLE CELL OF NECTURUS. By Albert C. Eycleshymer.

The work was done under the direction of Prof. Charles S. Minot and while the yriter held an Austin Fellowship in the Harvard Medical School.

On the nuclear changes during certain phases of cell life we possess an extensive literature, but concerning the *rôle* of the nucleus in histogenesis we know but little.

For the study of nuclear changes during histogenesis there is probably no cell more suitable than the striated muscle cell, since it is here possible to determine, not only volumetric relations, but also periods of maximal cytoplasmic activity as revealed by fibrillation. The investigations have led to the following tentative conclusions: the nuclei undergo striking changes in position, in that they migrate to areas of accelerated cytoplasmic activity.

The amount of chromatin in a given nucleus is increased pari passu with increased cytoplasmic activity. Further, it becomes unequally distributed in the nucleus, showing a marked condensation on the side which is applied to the fibrillated tract. This increase in the amount of chromatin is to be interpreted as an elaboration of nuclein which is given off and later finds its way into the dark band of the fibril.

In the earliest phases of differentiation a unit of nuclear material is in physiological equilibrium with two to three units of cytoplasmic material, while in the adult a unit of nuclear material is equilibrated with twenty to thirty units of cytoplasmic material. Before regeneration is possible, this disproportion is corrected by an increase in the amount of nuclear material.

Nuclear differentiation accompanies cytoplasmic differentiation, the nuclei of the different tissues showing structural and chemical differences.

THREE ANOMALIES OF THORACIC BLOOD-VESSELS. By Valray P. Blair.

1. Anomalous Pulmonary Vein.—This was observed in an adult male who died of pneumonia. No other history obtainable. The anomaly is a vein arising from the superior lobe of the left lung near its root. Its size is little less than that of an ordinary pulmonary vein. It empties into the left brachio-cephalic vein. The vein could be traced almost down to the base of the lobe running in the substance of the lung near the mesio-ventral border. It seems to be a true pulmonary vein and its capacity would indicate that about one-sixth of the blood was returned from the lung to the right side of the heart. (A description of this anomaly will appear in the Medical Bulletin of the Washington University, St. Louis.)

2 and 3. Anomalous Subclavian Arteries.—In both instances the right subclavian artery had its origin from the left side of the arch of the aorta, the one from the convexity, the other from the concavity of the arch.

TWO SPECIMENS OF ANOMALOUS VISCERA WITH LEFT-SIDED APPENDIX. By Edmund W. Holmes.

Read by title.

SITUS VISCERUM INVERSUS. By Robert J. Terry.

This complete transposition of viscera was found at the autopsy of a man 20 years of age, American, who died of appendicitis. His family knew of his peculiarity. He was not a twin, and was right-handed. Rotation of the gut took place from right to left, as evidenced by the right vagus nerve going to the front of the stomach.

The right lung is two-lobed; the left one has three lobes and is short and heavy.

The spleen is not lobulated and accessory spleens have not been observed. It may be worthy of note that the right testicle was lower than the left.

MODELS OF THE HUMAN PHARYNX OF THE FIRST SIX WEEKS OF DEVELOPMENT. By Merwin T. Sudler. See *American Journal of Anatomy*, Vol. I.

Read by title.

MORPHOLOGY OF PYLORIC GLANDS AS SHOWN BY RECONSTRUCTION. DEMONSTRATION OF MODELS. By Lydia M. DeWitt.

Until quite recently our knowledge of the morphology of microscopic objects depended on the study of sections and of teased preparations. The reconstruction methods of His and Born have added materially to our knowledge.

My work includes the reconstruction, by the Born plate method, of the pyloric glands of man, dog, cat at various stages of development, rabbit, turtle and frog. These glands in the dog are in the main tubular in type, richly branched and convoluted, with occasional enlargements resembling alveoli. In the cat the glands are tubular, convoluted and generally but little branched, but occasionally showing repeated division of the tubules. In the rabbit the glands represent long, slender tubules, slightly twisted and but little branched, but several often opening into a single crypt. In the turtle the pyloric glands are short and thick and have a distinct branched tubular type. In the frog these glands are simple tubular, several at times opening into a single crypt, but otherwise showing little tendency to branching.

The work will also include the reconstruction of Brunner's glands of the duodenum with the view of comparing and determining the relationship of these two types of glands.

THE SPHINCTER SUPERIOR. By Robert C. Bourland.

There is on the inner surface of every rectum a transverse fold extremely variable in size and generally situated upon the posterior and right lateral wall about six centimeters above the anus. This structure is the principal valve described by Houston. While the single fold is the condition by far most frequently met with, there may be another fold from one to four centimeters above the first on the opposite side of the gut. When this condition exists, the two folds are generally united, forming one spiral fold completely enclosing the intestine. Other transverse folds above and below are sometimes, though rarely, met with.

This constantly occurring valve consists of a fold of mucous membrane enclosing muscularis mucosæ, submucosa, and as a rule a thickened portion of the circular muscular coat. The circular muscular coat always presents a very distinct thickening, generally situated within the valve and forming the greater part of the tissue constituting the base of the valve; occasionally this thickening is found in the immediate vicinity of the valve.

The most important structural elements of the valve are therefore the fold of the mucous membrane and the above mentioned thickened muscular coat and these need to be considered in determining the significance of this fold.

By reason of the fact that an increase of the circular muscular coat is always associated with this valve, partly surrounding the intestine and sometimes, in conjunction with another fold, completely surrounding it, I feel warranted in the conclusion that the muscle is of primary importance and that the principal valve of Houston represents imperfectly a third or superior sphincter.

THE DUCTS OF THE HUMAN SUBMAXILLARY GLAND. By Joseph Marshall Flint. *American Journal of Anatomy*, Vol. I.

THE PANCREATIC DUCTS IN THE DOG. By Daniel G. Revell. *American Journal of Anatomy*, Vol. I.

VARIATIONS IN THE DISTRIBUTION OF THE BILE DUCTS OF THE CAT. By Roswell Hill Johnson.

This paper is a preliminary account of 85 out of a proposed 100 cases. Since a complete analysis will shortly be published only a few of the principal determinations will be given here.

In 7 cases there was one hepatic duct as in man. In a plurality of

cases (35) there were two hepatic ducts, but in a majority more than two, there being 26 with three, eleven with four, three with five and one each with six, seven and nine hepatic ducts. The duct from the Spigelian lobe which was most variable was found leading into ten different ducts. The variation was so great that only 3% were typical, *i. e.*, had every duct conform to the most usual condition of that particular duct.

DEVELOPMENT AND VARIATIONS IN THE DISTRIBUTION OF THE THORACICO-ABDOMINAL NERVES. By CHARLES R. BARDEEN. *American Journal of Anatomy*, Vol. I.

THE FRONTAL FISSURES IN THE BRAINS OF TWO NATIVES OF BRITISH NEW GUINEA. By GEORGE S. HUNTINGTON.

The brains of two natives of British New Guinea are probably of the Papuan race. The fissural pattern of all four hemicerebra is of a very simple and apparently fundamental type. The paper dealt with the fissures and gyres of the frontal region, and especially with the value and position of the medifrontal fissural element. The examination of the four hemicerebra throws some light on the probable derivation of this fissure, and on the consequent arrangement of the frontal gyres.

CONTRIBUTION TO THE ENCEPHALIC ANATOMY OF THE RACES. By EDWARD A. SPITZKA.

The intellectual characters of the races exhibit remarkable differences, and since they are but the expressions of cerebral activity, the assumption that in the brains of different races and nationalities exist typical differences of cerebral surface morphology, is a belief that seems to be rendered justifiable by even the meager amount of material that has so far accumulated. What is to be attained in this view is the establishment of a systematic anthropological encephalotomy. The greatest hindrance to the pursuit of such work lies in the difficulty of procuring the necessary material. The present series of papers comprises three Eskimo brains from Smith's Sound, one Japanese brain and the brains of two female natives (Papuans?) from British New Guinea. It is hoped to extend the series whenever the required material becomes available for study. These brains are exceedingly rare specimens. The three last mentioned seem to be unique in anatomical literature, and only four other Eskimo brains have been described so far—three by Chudzinski in 1881, the specimens being in a bad state of preservation, and one by Hrdlicka in 1899, a fine specimen from an

Eskimo chief named "Kishu." The three brains here described belong to Kishu's tribe. A preliminary report has been presented by Prof. G. S. Huntington to this Association in May, 1897, on the two Papuan (?) brains.

DESCRIPTION OF THE BRAIN OF A REGENTICIDE. By Edward A. Spitzka.

Illustrated by drawings made from nature of the brain of Leon F. Czolgosz, the assassin of President McKinley, also by photographs of a plaster cast of the entire head, and by outline drawings of the skull.

E. A. Spitzka, who performed the autopsy upon assassin Leon F. Czolgosz, was fortunate enough to be able to make a plaster cast of the entire head, as well as drawings of the outer features of the brain and skull. A full description of the cerebral fissures and convolutions was recorded stenographically. The autopsy revealed no evidence whatever of disease or deformity of any of the bodily organs, including the brain, which was normal in size, shape, weight, and appearance. The assassin was in excellent health at the time of his death, and the post-mortem findings corroborate positively the opinion formed by all the alienists who examined the prisoner, that he was entirely free from mental disease and fully responsible for his deed. The reader will find the full report, with the anthropometric measurements and illustrations of the cast, brain and skull in the leading medical journals published January 4, 1902.

AN ILLUSTRATION OF THE VALUE OF THE FUNCTIONAL SYSTEM OF NEURONES AS A MORPHOLOGICAL UNIT IN THE NERVOUS SYSTEM. By C. Judson Herrick.

The peripheral gustatory system of neurones was briefly reviewed in the vertebrate series. The fibers have probably been derived from unspecialized visceral sensory fibers, the fibers of both the unspecialized visceral and the specialized gustatory varieties comprising the *communis system* of the cranial nerves as known to comparative anatomy. This system (both kinds of fibers) is typically represented in the X, IX and VII pairs of cranial nerves, but it is subject to very remarkable variations depending on the number and distribution of the tastebuds. Some of these variations were passed in review and emphasis laid on the permanence of the morphological plan of the communis system throughout the series. The phylogenetic history of the facial nerve was briefly sketched from the same point of view.

CONTRIBUTION TO THE MORPHOLOGY OF THE CEREBELLUM. No. IV. VARIATIONS OF THE HUMAN LINGULA. By Bert B. Shroud.

The commonly accepted view of the *lingula cerebelli* is "a group of four or five transverse laminæ which lie upon the middle of the valvula (velum medullare superius)."

This definition is true in some cases, but not in all.

1. There may be as many as seven transverse ridges.
2. One of the ridges may be developed into a foliated lobule.
3. There may be only a thin layer of ectocinerea with no ridges and a foliated lobule.

ON THE CRANIAL ANATOMY OF THE PLESIOSAURUS. By S. W. Williston.

The study of a remarkably complete skull of a plesiosaur, recently discovered in Kansas, has disclosed a number of interesting new facts in the cranial anatomy of this order of reptiles. The brain cavity is relatively large, as are also the semicircular canals; the alisphenoid is not ossified; the paroccipital (opisthotic) is completely fused in the young skull; and the supraoccipitals are parial, separated by a broad vacuity, containing the foramen magnum to the parietal. The single temporal arcade is composed of squamosal, prosquamosal, jugal and quadratojugal, representing both arcades of other reptiles. The frontals are separated in their whole length by an anterior prolongation of the parietals. There is no separate post-orbital, lachrymal or nasal, the last evidently fused with the parietal in the adult skull. The anterior or membranous portion of the articular of most reptiles exists as a separate ossification, confirming Baur's homologies of the reptilian mandible. There is a complete and well-developed ring of sclerotic plates in the orbits.

The epipterygoid joins both parietal and frontal above, in all probability. In three genera of plesiosaurs studied by the author, a distinct oval foramen was found back of the interclavicle and between the clavicles, which may be called the interclavicular vacuity. He believes that the interclavicle is of membranous origin and hence cannot be the epi- or omosternum.

HISTOGENESIS OF THE SENSORY NERVES OF AMPHIBIA. By Ross G. Harrison.

ON THE NUMBER AND SIZE OF THE SPINAL GANGLION CELLS AND DORSAL ROOT FIBERS IN WHITE RATS OF DIFFERENT AGES.

By Henry H. Donaldson.

Dr. Donaldson presented some results obtained by Mr. Hatai from his studies on the spinal ganglion of the white rat during the growing period.

Four rats were used, their body weights being 10, 24, 68, and 167 grams, respectively. In each rat the number of cells in the ganglion and the number of fibers in the dorsal nerve root of the sixth cervical, fourth thoracic, and second lumbar was determined. On comparing the number thus obtained, it was possible to draw the following conclusions:

1. In the spinal ganglia the number of cell bodies is constant between birth and maturity; there is, of course, some individual variation in this number.

2. The fibers of the dorsal nerve roots are more than twice as numerous in the 167-gram rat as in the 10-gram rat, and the intermediate weights show intermediate numbers in the roots.

3. Since this is the case, it follows that the ratio between the number of cells in the ganglia as compared with the number of fibers in the dorsal nerve root steadily decreases. In the youngest stage, there may be as many as eleven cells in the ganglion for each fiber in the dorsal nerve root, while in the case of the most developed nerve, there are still 2.7 cells for each fiber.

It appears that the new fibers are formed by the outgrowths of cells present in the ganglion from the earliest stage.

Studies on the general activity of the rat show that they are most active when weighing from 25-35 grams, and therefore, at a time when the number of fibers in the dorsal nerve roots is still very incomplete.

THE NEUROGLIA OF THE OPTIC NERVE AND RETINA OF CERTAIN VERTEBRATES.

By G. Carl Huber.

These observations embrace a study of the structure and distribution of the neuroglia of the optic tract, chiasm of the nerve and retina of the dog, cat, dove, tortoise, and frog, and also fragmentary observations on the neuroglia of these structures as found in man.

In this study, Benda's recently published method for staining the neuroglia was used; this the writer has found applicable for staining the neuroglia of vertebrates other than man.

NOTE ON THE STRUCTURE OF THE MOTOR NERVE ENDINGS IN VOLUNTARY MUSCLE. By G. Carl Huber.

In three articles recently published by Sihler, dealing with the termination of nerves in muscle tissues, he has criticised certain observations made by Huber and DeWitt, the discussion having reference to the relation of the sarcolemma to the motor endings. In our work we stated that the endings were hypolamellar. Sihler contends that they are external to the sarcolemma, he basing his statements on observations made on tissues stained by his acetic acid-hæmatoxylin method, while our conclusions were based on the study of tissues stained *intra vitam* with methylene blue, fixed, sectioned, and counterstained in alum carmine. In striated muscle, fixed and hardened in bichloride and stained after Mallory's aniline blue-fuchsin connective tissue stain, the connective tissue and sarcolemma stain blue, while the muscle stains red or orange red. It seemed therefore probable that this method might be used to advantage in ascertaining the relation of the sarcolemma to the motor ending. One of the small intrinsic plantar muscles of a rabbit's foot was fixed in bichloride solution, cross sections of 3 μ or less in thickness were made and stained after the above mentioned method. When such sections were studied under the 1/12 in. oil immersion with the No. 12 compensation eye-piece (Zeiss), numerous motor nerve endings were observed, in which there seemed no question but that the motor nerve ending in voluntary muscle is under the sarcolemma.

NEURO-MUSCULAR SPINDLES IN THE INTERCOSTAL MUSCLES OF THE CAT. By G. Carl Huber.

That neuro-muscular spindles are found in the intercostal muscles is well known. The present paper deals with the number and distribution of the sensory nerve end organs in the intercostal muscles of the cat. The successive intercostal muscles of one side were removed and stained after Sihler's acetic acid-hæmatoxylin method. The tissue thus stained was separated into small pieces, each of which was crushed between two slides and the number of the sensory nerve end organs in each piece noted. In the six upper intercostals, from 60 to 100 sensory nerve endings were found in each intercostal space, from the 7th to the 10th they were somewhat less numerous, and in the 11th and 12th, 28 and 18 sensory nerve endings were found, respectively. Serial sections of the muscular tissue in the 4th intercostal space were made; no special grouping of the sensory nerve endings was observed, and it was found

that they were about equally distributed in the external and internal muscles. Attention is called to the relatively large number of these sensory nerve end organs in these muscles. The suggestion is made that they initiate reflexes which form one of the factors necessary for maintaining the respiratory rhythm.

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In closing the first volume, it is gratifying to be able to record an exceptionally encouraging reception, and to feel justified in anticipating a constant and growing support.

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